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OXYGEN CONSUMPTION OF PERFUSED OMASAL LAMINAE

by

© FREDERICK JOHN LOZEMAN

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
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IN
ANIMAL BIOCHEMISTRY

DEPARTMENT OF ANIMAL SCIENCE

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THE UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled OXYGEN CONSUMPTION OF PERFUSED OMASAL LAMINAE submitted by FREDERICK JOHN LOZEMAN in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in ANIMAL BIOCHEMISTRY.

ABSTRACT

The vascular anatomy of the omasal leaf permitted development of a technique in which an isolated area of the tissue could be perfused and monitored for O_2 consumption. Perfusion of a discrete area of an omasal leaf effectively allowed the provision of O_2 to the omasal epithelium during the maintenance of anaerobic conditions on the lumen side of the tissue. This condition represents the physiological situation in the ruminant animal, in which the forestomach epithelium constitutes the interface between the anaerobic gut and the animal tissues.

Two series of experiments were performed on bovine omasal leaves collected from a local abattoir. Endogenous rates of O_2 utilization in the two experiments were 64.9 ± 7.85 and 73.5 ± 6.41 nmoles O_2 (mg dry weight)$^{-1}$ h^{-1} . Over the course of both experiments, exposure of the tissue to an atmosphere of N_2 resulted in a consistent and dramatic elevation in O_2 consumption above the endogenous rate ($p \leq 0.05$) to 87.4 ± 15.70 and 104.3 ± 9.19 nmoles O_2 (mg dry weight)$^{-1}$ h^{-1} respectively.

In the first experiment, a boiled suspension of rumen particles (≤ 1 mm) and microorganisms placed into the lumen chambers significantly increased mean O_2 consumption rates ($p \leq 0.05$). Replacement of the boiled suspension with a similarily prepared unboiled suspension increased mean O_2 consumption by a further 11%, but this increase was not statistically significant ($p > 0.05$). Addition of butyrate (8

mM) and propionate (22 mM) to the unboiled rumen particle suspension did not invoke any further response in O_2 consumption.

In the second series of experiments, butyrate (8 mM), propionate (22 mM), and D,L-3-hydroxybutyrate (8 mM) were added sequentially into the anaerobic lumen solution. Butyrate stimulated a 31% increase ($p \leq 0.05$) in O_2 consumption but the subsequent additions failed to induce further increases. Replacement of this solution with a boiled, suspended preparation of strained and washed rumen contents (plus the substrates listed above) resulted in an additional 18% increase ($p \leq 0.05$) and substitution of the boiled suspension with an unboiled one contributed a further 12% increase ($p \leq 0.05$) in O_2 consumption rate.

Thus O_2 withdrawal from the perfusate, as it passed through the tissue, was stimulated by the presence of a concentrated suspension of rumen particles and microorganisms on the lumen sides of the tissue. The components involved in stimulating the removal of O_2 were not positively defined, however there seems to be both a heat-stable and a heat-labile component. The most obvious heat sensitive fraction of the suspensions is the microorganisms, which may either promote greater tissue utilization of O_2 or may extract O_2 for use in their respiration. Results and observations also indicate substantial diffusion of O_2 out of the tissue, since perfusate saturation of O_2 could be reduced during lumen

chamber conditions which should not stimulate tissue respiratory activity. This diffusion is consistent with the theory that O_2 may be available to bacteria in close proximity with the forestomach wall; the bacteria may even stimulate greater provision of O_2 at the tissue - lumen interface.

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I. INTRODUCTION

Continuing research on metabolism in domestic ruminants has centered upon quantitating processes within the reticulorumen and within body tissues in order to determine the fate of nutrients and the efficiency of utilization of nutrients within the animal. More detailed investigation has shown that these factors are subject to many influences and interactions within the animal, some of which include physiological state, level of feed intake, chemical composition of the ration, physical state of the ration, etc. For instance, increasing the level of feed intake may result in a decreased apparent digestion of organic matter in the rumen (Sutton 1980) but may also increase the efficiency of microbial growth due to an increase in rumen dilution rate (Kennedy and Milligan 1978; Harrison and McAllan 1980; Hobson and Wallace 1982b). Sheep exposed to moderate cold exhibited substantially larger portal blood absorption of propionate than sheep in a thermoneutral environment fed the same diet at the same level of intake (Thompson et al. 1978).

Therefore it is evident that many factors within the animal may interact to determine the digestion, absorption and metabolism of nutrients. Recent observations related to the functioning of the forestomach epithelium indicate that this tissue may be involved in the interacting factors which affect utilization of nutrients. Substantial epithelial sloughing into the forestomachs (Kennedy and Milligan 1980b)

suggests that the forestomach epithelium may require a substantial amount of nutrient for maintenance. Changes in dietary intake and physical and chemical form of the diet may also exert effects on the mass of the tissue and on its nutrient requirement (Fell and Weekes 1975). Epithelial transfer of nitrogen into and out of the reticulorumen may be substantial depending on many interacting factors (Kennedy and Milligan 1980a) and evidence has suggested that the control of urea recycling into the reticulorumen is accomplished via urease activity associated with the epithelium (Cheng and Wallace 1979). The urease is bacterial in origin however, because a large population of ureolytic bacteria colonize the forestomach epithelium (Cheng and Costerton 1980), the control of urea recycling may be effected in the epithelium. The presence of a relatively large adherent bacteria population, of which 0.25 - 0.5 are facultative anaerobes (Cheng and Costerton 1980), also raises the possibility of an interaction between adherent bacteria and the epithelium. Cheng and Costerton (1980) and Cheng et al. (1981) have proposed diffusion of O_2 to the surface of the epithelium and subsequent utilization of O_2 by the adherent bacteria.

In light of these observations, related evidence and speculations, study of the forestomach epithelial metabolism and respiration is warranted. The forestomach mucosa could be a quantitatively important site of use of available nutrients and may require a substantial amount of substrate

for tissue maintenance. It may be involved in changing the flux of net metabolite absorption or recycling during lactation, cold stress, increased feed intake, etc. In addition, diffusion of O_2 from this well vascularized tissue to the lumen of the forestomach could have quantitatively important effects on microbial growth yields in the forestomachs. With this in mind, this investigation was developed for the assessment of respiration of forestomach epithelium in the presence of controlled, anaerobic conditions on the lumen side(s) of the tissue. A special emphasis was placed upon determining the effect of a concentrated population of rumen bacteria on the rate of tissue respiration and on the possibility of O_2 diffusion from the vascular system of the forestomach wall to the lumen surface.

II. BACKGROUND INFORMATION

The function of the ruminant forestomach is to provide an environment in which the population of rumen microorganisms can adequately perform their maintenance and growth functions, while at the same time it must benefit the animal which utilizes this pre-gastric type of fermentation in order to provide nutrients in a readily usable form. It is presumed that the structure of the forestomach epithelium is related to the specific function it must fulfill ie. to provide an interface that is suited to the animal as a whole and to the microorganisms which ferment the feedstuffs within the forestomach.

For many years, it was thought that the lining of the ruminant forestomach would not allow the permeation of any materials into and out of the rumen because of the keratinized composition and structure of the epithelium. Experimental work in the 1940's and 1950's did finally suggest permeability of the rumen wall to certain products of fermentation (Barcroft et al. 1944; Annison et al. 1957; Kiddle et al. 1951; Masson and Phillipson 1951). In hindsight, the ability of the forestomach epithelium to selectively allow passage of certain substances while excluding the passage of others is entirely logical, considering the function of pre-gastric fermentation. The epithelium must allow the removal of fermentation products which could cause a decrease in fermentation rate presumably by end-product inhibition (Ewart 1974). It must prevent loss

of valuable substrates for microbial metabolism as well as provide adequate protection from toxins present in the diet or generated in the rumen and protection from the penetration of rumen microorganisms into the body tissues.

A. Structure and development of the forestomach epithelium

Since there are several reviews of forestomach wall structure and organization (Hyden and Sperber 1965; Steven and Marshall 1970) and development of the forestomach epithelium with age and diet (Warner and Flatt 1965; McGilliard, Jacobson and Sutton 1965; Fell and Weekes 1975), discussion will be limited to aspects of epithelial structure and development which have an impact on transfer of materials through the epithelium and metabolism of materials as they pass through the epithelial layers.

As briefly mentioned above, the forestomach lining must not only be permeable, but it must be permselective. These properties are common to all epithelia with barrier function (see Powell 1981 for a discussion). Single-layered and multi-layered epithelia essentially have two routes of movement; 1) a transcellular route which involves the cell membranes as barriers and 2) a paracellular route which avoids movement through the cells but entails barriers at cell junctions and travel through intercellular space (Powell 1981). These properties are characteristic of the multi-layered keratinized epithelium of the forestomach.

Essentially, the forestomach epithelium has four cell layers (Steven and Marshall 1970):

stratum basale
stratum spinosum
stratum granulosum
stratum corneum

The stratum basale layer is adjacent to the basement membrane of the epithelium, while the stratum corneum is the outermost or peripheral layer (lumen). The stratum basale layer appears to be the most metabolically active since it has a substantial population of evenly distributed mitochondria and numerous free ribosomes (Steven and Marshall 1970). The major barrier to absorption seems to be on the luminal side of the active cells of the epithelium (Steven and Marshall 1970) and the most likely layer of cells providing the obstruction is the stratum granulosum. This stratum is characterized by very tight cell junctions and a complete lack of intercellular space (Steven and Marshall 1970) which suggests that this layer of cells may be able to exercise tight control on the movement of solutes from the lumen of the gut or into the lumen of the gut.

The construction of the forestomach epithelium prompted Stevens et al. (1969) and Steven and Marshall (1970) to consider the epithelium as a distinct compartment separating the lumen from the extracellular space of the epithelium.

Therefore any substances that were to be transferred from the lumen to the blood plasma would have to pass through at least one layer of epithelial cells, allowing strict control of permselectivity and rates of absorption.

Henrikson (1970) maintained a contrasting opinion from Steven and Marshall (1970). Basically, he maintained there were no tight cell junctions (zonulae occludentes) to provide a physical barrier to movement but rather the series of cell spaces, cell processes, desmosomes, maculae occludentes and the coating of mucopolysaccharide on the keratinized cells provided an "effective" barrier to movement of fluids and solutes (see also Fell and Weekes 1975). Regardless of the structure of the epithelium, the resultant effect is similar; a control of permeability and permselectivity.

Although gross microscopic (light or electron) examination of the epithelium in different areas of the forestomach does not reveal obvious differences between epithelial structure, recently Tamate and Sakata (1979) have observed the epithelium-propria interface of the forestomach mucosa by scanning electron microscopy (the epithelium-propria interface is the boundary between the epithelium and underlying connective tissue which is separated by the basement membrane; see Figure II.1). Well developed rumen papillae are characterized by an interface which exhibits well developed basal cells and a much greater surface area between the cells of the epithelium and the

underlaying connective tissue (Tamate and Sakata 1979). The phenomenon of this increased surface area at the epithelium-propria interface was referred to as the development of epithelial pegs (Sakata and Tamate 1978; Tamate and Sakata 1979). Epithelial pegs are also referred to as epithelial grooves (Cheetham and Steven 1966). Developed epithelial pegs and a relatively high number of stratum basale cells are indicative of the enhanced absorptive and metabolic activities of that specific area of the forestomach wall (Tamate and Sakata 1979). This was typical of mucosa that exhibited well developed papillae, while an epithelium-propria interface with relatively little development of epithelial pegs was typical of the mucosa of omasal laminae, ruminal pillars, reticular mucosa and the reticular and omasal grooves (Tamate and Sakata 1979) which presumably were not as efficient in absorption or as active in metabolism (see Figures II.1 and II.2).

The factors which control development of the mucosa are not well understood, although it is evident that the development of an active rumen fermentation in young ruminants will stimulate the absorptive ability and metabolic activity of the forestomach mucosa (Sutton et al. 1963a,b; Sander et al. 1959). A review of the literature up to 1964 (McGilliard et al. 1965) left little doubt that the accumulation of end-products of microbial fermentation triggers the development of structural and metabolic maturity in the forestomach mucosa of young ruminants. The

continued feeding of an exclusively milk diet in calves up to 13 weeks of age and in one calf up to 34 weeks of age had no effect upon absorption of acetate from the rumen (Sutton et al. 1963a). Alternatively, calves introduced to a diet of milk, hay and calf starter exhibited an almosty twenty fold increase in the rate of acetate absorption from the rumen after 13 weeks (Sutton et al. 1963a). This and numerous other studies (Sander et al. 1959; Tamate et al. 1962; Walker and Simmonds 1962; Sutton et al. 1963b) have established that VFA seemed to be the most potent stimulus for epithelial development. Thus it is tempting to postulate that the epithelium requires VFA as its major energetic and synthetic substrates, although glucose may be required for cell mitosis (Fell and Weekes 1975).

Steven and Marshall (1970) observed that the cell junctions of the forestomach epithelium in the newborn calf are almost fully developed while the internal organization of the epithelial cells must undergo extensive development in order to resemble the organization seen in the adult forestomach epithelium. In other words, the barrier function of the epithelium is in place at or before birth, but the absorptive ability of the epithelium must be stimulated by development of the intracellular organization of the epithelial cells. How the intracellular development augments the absorptive ability of the tissue is not known, however, it could be related to metabolism within the epithelial cells and/or the degree of development present at the

epithelium-propria interface since an increased rate of mitosis is related to a higher frequency of epithelial pegs and, therefore, to a more absorptive mucosa (Sakata and Tamate 1978; Tamate and Sakata 1979). In one of these studies (Sakata and Tamate 1978) the periodic intraruminal administration of butyrate (18 mmoles kg BW^{-1} day^{-1}) induced an increased proliferation of epithelial cells, especially the cells of the stratum basale layer. The epithelium of sheep administered butyrate once daily in a 10 second interval exhibited hypertrophy of epithelial pegs, suggesting improved absorptive capacity as compared to the sheep infused continuously with the same amount of butyrate over the whole day. A subsequent study with acetate or propionate administered intraruminally (18 mmole kg BW^{-1} day^{-1}) in one 10 second interval during the day also resulted in a significant increase in the mitotic index of the rumen epithelium (Sakata and Tamate 1979) but those VFA were not as potent as butyrate.

The activity of several cytoplasmic enzymes which participate in generation of NADPH are much higher in rumen epithelium than in liver tissue. Young et al. (1969) found 40 to 240 fold more activity of malic enzyme (EC 1.1.1.40) in rumen epithelium than in liver. This was corroborated by Whanger and Church (1970), who also showed that malic enzyme activities in rumen epithelium and small intestine mucosa were similar. In addition, activity of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) in rumen epithelium was 4 to 8

times greater than in liver (Young et al. 1969) while 6-phosphogluconate dehydrogenase (EC 1.1.1.44) was equally active in rumen epithelium, liver and small intestinal mucosa (Whanger and Church 1970). The presence of enzymes and pathways which generate reduced dinucleotides indicate a demand for reducing power for cellular synthetic functions.

The exact mechanism by which VFA cause hyperplasia of rumen epithelium is largely unknown, but action of absorbed VFA on the endocrine system cannot be overlooked. Sakata and Tamate (1979) implicated insulin in the process, following stimulation of release of insulin by VFA. Direct control of VFA on epithelial cell intermediary metabolism may also regulate epithelial cell mass.

A discussion of the structure and growth of epithelium must also consider the sloughing of the luminal layers of cells. Certainly, areas of mucosa subject to more abrasive action of the gut contents require more rapid replacement of sloughed cells than other areas not exposed to such harsh abrasion. Fell and Weekes (1975) maintained that the relative thickness of the rumen epithelia may be determined by the amount of cell sloughing, as well as the rate of mitosis in basal layers and the time required for basal cells to migrate to the keratinized layer. Nocek et al. (1980) made some very interesting observations on the transport of acetate and propionate across isolated rumen epithelium of young calves that may illustrate the importance of cell sloughing. They found that mucosa taken

from 20 week old calves on a concentrate diet exhibited significantly heavier ruminal epithelial tissue dry weights and significantly lower rates of acetate and propionate transport across isolated sheets of stripped rumen epithelium (rumen wall in which muscle was separated from the epithelium/subepithelium) as compared to mucosa taken from calves on a ground or chopped hay diet. Although the tissue was not examined microscopically, the authors speculated that there was an accumulation of degenerate, outer-layer tissue, with the concentrate diet. This diet provided readily available substrates, which would stimulate rumen epithelial cell proliferation, by means of large increases and fluctuations of VFA production but may not accelerate the loss of the keratinized layer of cells due to the lack of an abrasive component in the diet (Nocek et al. 1980). Nocek et al. (1980) claimed that the thickened stratum corneum layer resulted in reduced VFA transport rates because it provided a larger barrier to diffusion. Weigand et al. (1975) considered this explanation in their experiment on grain-fed and roughage-fed steers but they suggested the more likely possibility that, because of a proliferation of the less metabolically active outer cell layers of the epithelium, an expression of VFA transport or metabolism per unit weight or per unit N would consequently be depressed (Weigand et al. 1975). However, it should be stressed that even though transport or metabolism of VFA expressed on a per unit weight basis may be slightly

depressed in mucosa of concentrate-fed animals, the overall absorptive ability of the tissue may be increased due to more papillary development, larger epithelium-propria surface area, more metabolically active cells, etc. Thorlacius and Lodge (1973) reported a more rapid absorption of VFA from the washed ventral sac of a cow fed a concentrate diet as opposed to a hay diet, despite an apparent hyperkeratinization of the ruminal mucosa.

The contribution of bacteria adhering and colonizing the peripheral cells of the forestomach epithelium must not be overlooked, since they may in fact accelerate the digestion of this keratinized layer of epithelial cells (Cheng and Costerton 1980). However, investigation of distribution patterns of these adherent bacteria in the bovine reticulorumen suggest that the bacteria may be susceptible to the abrasive action of the food (McCowan et al. 1980) in a manner analogous to that observed on epithelial cells. Thus the digestion of epithelial tissue by bacteria would be more significant in areas of greater colonization; ie. areas of the forestomach wall which experience less abrasion from feedstuffs.

Although evidence seems to indicate that forestomach epithelium is underdeveloped structurally, metabolically the epithelium from young ruminants may be more active than the tissue from more mature ruminants. In vitro measurement of O_2 consumption of underdeveloped rumen epithelium (from 2 week old lambs) consistently exhibited a higher respiration

rate than epithelium from lambs which were 6 months old (Giesecke et al. 1979). However, epithelium from the older lambs seemed to attain a higher preference for butyrate as a substrate as opposed to glucose or lactate which were preferred substrates for the underdeveloped epithelium (Giesecke et al. 1979). It should be noted that the epithelium obtained from 8-12 week old lambs fed a strictly milk diet did not undergo the extent of hyperplasia observed for similarly aged lambs placed on a hay concentrate diet, but neither did it exhibit a significantly different respiration rate, with or without, glucose as a substrate (Giesecke et al. 1979). These observations tended to indicate to the authors that the metabolic activity (as measured by O_2 consumption per unit dry weight and time) of rumen epithelium was high at birth, and has a tendency to decrease with age but not diet. Alternatively, epithelial development (as measured by increased epithelial thickness) and preference for substrate did seem to respond to the type of feeding. The authors speculated that the increased epithelial thickness and increased preference for butyrate as a substrate was indicative of a lesser dependance on glucose as the fermentative ability of the rumen was developed (Giesecke et al. 1979).

In summary, forestomach epithelium responds to increased feed intake, increased levels of highly fermentable substrate in the forestomach, and any other factors which may increase VFA production rates (especially

butyrate), by proliferating epithelial cells of the basal layers and thereby resulting in a more developed epithelium-propria interface. The consequence of this structural alteration in the epithelium is presumably, a more absorptively efficient tissue. The mechanism of control of the epithelial hyperplasia and atrophy remains obscure, but is obviously the result of fine control on cell mitosis, cell necrosis (or apoptosis) and rate at which the cells become keratinized and eventually sloughed.

B. Absorption from the forestomach

Brief mention will be allotted to absorption of substances through the forestomach epithelium. For a general discussion of absorption of quantitatively important fermentation products and by-products, the reader is referred to Dobson and Phillipson (1968), Church (1976) and Bergman (1975).

Absorption of volatile fatty acids

The earliest indication of VFA absorption from the forestomach was provided by appearance of VFA in blood draining the rumen (Barcroft et al. 1944). Similar observations subsequent to this, coupled with measures of disappearance from the reticulorumen and omasum, have furnished the best estimates of in vivo absorption via the forestomach epithelium. These studies have indicated that 0.85 of VFA production in the reticulorumen is absorbed from

this organ (Whitelaw et al. 1970; Engelhardt and Hauffe 1975; Endrise and Smith 1977, 1979) and 0.10 of production is absorbed from the omasum (Engelhardt and Hauffe 1975; Endrise and Smith 1977, 1979). Estimates of propionate outflow from the rumen of a sheep infused with propionate indicated 0.81 to 0.83 of total propionate production was absorbed from the reticulorumen and 0.56 to 0.59 of the total appeared in the portal blood (Weekes and Webster 1975).

In vivo rates of VFA disappearance from the emptied and washed bovine reticulorumen at a given pH and physiological concentrations of the three major VFA are consistently measured in the order of acetate>propionate>butyrate (Sutton et al. 1963a; Weigand et al. 1972b; Thorlacius and Lodge 1973). However, specific absorption rates (which are rates of disappearance relative to the initial concentration of the specific VFA) are butyrate>propionate>acetate (Sutton et al. 1963a; Weigand et al. 1972b).

Studies performed with isolated sheets of rumen epithelium have established that VFA absorption is a passive process which is largely controlled by metabolism within the epithelial cells, prevailing pH of the lumen (and presumably of the cells and extracellular fluid), and electro-chemical gradient (Stevens and Stettler 1966a,b; Stevens 1970). Stevens (1970) maintained that all of these factors exert effects on absorption because the forestomach epithelium acts as at least one separate compartment between the lumen

and the extracellular space of the tissue.

Absorption of ammonia

The fact that ammonia is a very important intermediate of nitrogen metabolism in the ruminant makes the study of its transport to and from the forestomach highly relevant. As with VFA, first evidence of ammonia absorption from the forestomachs was provided by net appearance in ruminal venous blood (McDonald 1948). More quantitative estimates of ammonia absorption have been obtained through the use of ^{15}N isotope dilution studies. Nolan (1975) calculated that for a sheep on a 800 g day^{-1} lucerne diet, about one-half of the total flux rate through the rumen fluid ammonia pool was absorbed from the reticulorumen and omasum. Similarily, Mathison and Milligan (1971) reported that on a high protein diet, 0.71 of the ammonia production rate in the rumen of a sheep was absorbed from the rumen. These represent significant ammonia absorption rates of 4.4 and 7.2 g N day^{-1} respectively.

Ammonia absorption from the lumen of the gut is considered to be a passive process (Annison 1965) and thus it is largely dependant on the concentration in the rumen. In general, the more concentrated ammonia is in the reticulorumen, the higher the rates of absorption (Mathison and Milligan 1971; Kempton et al. 1979). In addition, there is a strong relationship between the concentration of unionized ammonia in the rumen and amount of ammonia

absorbed from the organ (Nolan and Leng 1982). In fact, rumen pH may be the main factor controlling rate of ammonia absorption at any given concentration (Nikolic et al. 1980).

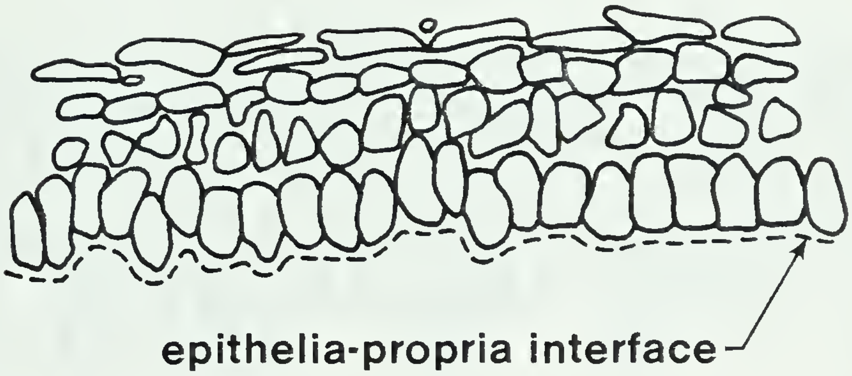

epithelia-propria interface

Figure II.1 Underdeveloped epithelial pegs in forestomach epithelium

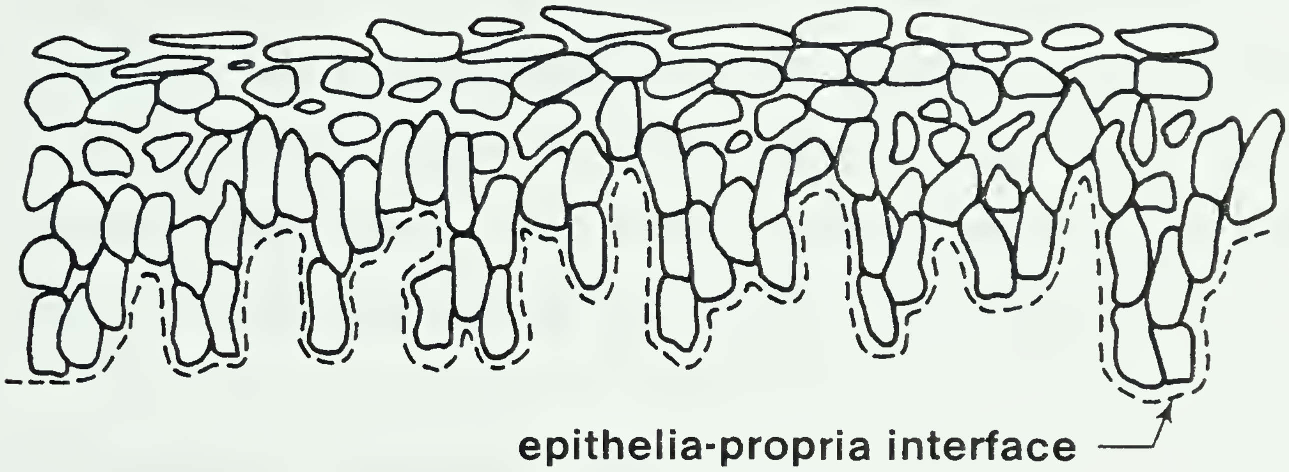

epithelia-propria interface

Figure II.2 Developed epithelial pegs in forestomach epithelium

III. METABOLISM WITHIN FORESTOMACH EPITHELIUM

Metabolism within forestomach epithelium contributes to the integration of metabolism in the entire animal, including metabolism within the gastro-intestinal tract. Therefore, it is the intent of this section to refer to aspects of the metabolic activity of the forestomach epithelium and to investigate possible substrates and products of metabolism in this tissue. Since many studies of metabolism in the gut tissues of ruminants are not centered upon deriving specific information on forestomach epithelial metabolism, much of the emphasis will be placed upon comparison of the epithelium of forestomach wall with other sections of the portal-drained viscera (which include the stomach compartments, pancreas, spleen, small intestines, large intestines, ceacum and part of the rectum).

A. Metabolic activity

Blood flow to the forestomach epithelium

The degree of vascularization or volume of blood delivered to a specific volume or weight of tissue has often been associated with metabolic demand (Guyton 1971). Blood flow to the forestomach wall may indicate activity of the forestomach epithelium, since 95% of the capillary blood flow to the rumen wall is actually directed to the capillary bed of the forestomach subepithelium (Engelhardt and Hales 1977). In sheep that had not been fed for 18 h, the mean

capillary blood flow to the whole forestomach was estimated at 7.7 ml kg BW^{-1} min^{-1} (Engelhardt and Hales 1977). Portal blood flows in sheep of similar weight (also starved for 18 h before measurement) averaged 33 ml kg BW^{-1} min^{-1} (Katz and Bergman 1969; Webster and White 1973). Thus capillary blood flow to the forestomach walls constitutes 0.23 of total portal blood flow in the unfed sheep. However, attempts to estimate total blood flow to the forestomach organs have indicated that much of the blood to these organs must pass through arteriovenous anastomoses. From previous observations, Engelhardt and Hales (1977) estimated that total ruminal flow was 19 ml kg BW^{-1} min^{-1} . Mathison (1972) measured right ruminal venous blood flow rates of 10-53 ml kg BW^{-1} min^{-1} in sheep fed on various diets, which suggests a large blood flow to the rumen since the right ruminal vein of the sheep drains only 35-45% of the reticulorumen area (Dobson and Phillipson 1968). Hecker and Nolan (1971) and Engelhardt and Hales (1977) suggested that over 50% of portal blood flow is supplied by drainage from the forestomachs.

Presumably, a large portion of the blood directed toward the forestomach organs is shunted away from the capillary beds, but it is the capillary blood flow in the wall of the gastrointestinal organ that should reveal the best estimates of comparitive metabolic activity. Hales (1973a,b) disclosed capillary blood flow rates of 66-72 ml (100g $tissue)^{-1}$ min^{-1} and 128-130 ml (100g $tissue)^{-1}$ min^{-1}

in the ruminal wall and small intestinal wall respectively. (Note that the wall of the organ pertains to the mucosa, submucosa and muscle.) This may indicate that on a per tissue weight basis, the small intestinal mucosa exhibits a greater metabolic activity than the forestomach mucosa, although a greater proportion of the blood flow to the mammalian small intestinal wall may be diverted to the muscularis than is the case for the forestomach wall (Granger et al. 1980; Engelhardt and Hales 1977). Alternatively, relative capillary blood flows to the forestomachs, small intestine and hind-gut of sheep have been reported to be similar to each other (Schaeffer and Young 1980).

Christopherson et al. (1980) found significant drops in capillary blood flow of the rumen, reticulum, omasum, abomasum and duodenum of sheep after a 24 h fast. Increases in portal venous blood flow with feeding and for several hours after feeding are well documented (Bensadoun and Reid 1962; Webster and White 1973; Webster et al. 1975). Therefore, blood flow to the organs of the gastrointestinal tract are affected by feeding, however the contribution of individual digestive organs to the increment in portal venous blood flow is unknown. There is tentative evidence indicating that blood flow through the forestomach organs may account for relatively more of portal venous blood flow in the fed state. Sellers (1965) reported that arterial blood flow to the rumen of a cow increased 25-30% in

response to feeding while blood flow to portions of the lower intestinal tract were unaffected. Rumen arterial and subepithelial blood flows increased with elevated pCO_2 in the rumen (Sellers 1965; Dobson 1979) and a similar effect was observed in ruminal and omasal arteries with increasing levels of VFA in the rumen (Sellers 1965).

Oxygen utilization and heat production by forestomach and portal-drained tissues

In vitro oxygen consumption by bovine and ovine ruminal epithelium stripped of muscle and in the presence of butyrate are consistently measured at about 200 nmoles O_2 (mg dry weight)$^{-1}$ h^{-1} (Pennington 1954; Goosen 1976; Giesecke et al. 1979). Oxygen uptake by slices of ovine jejunal mucosa which had been stripped of muscle and incubated in the presence of 10 mM glucose, were measured at 230 nmoles O_2 (mg dry weight)$^{-1}$ h^{-1} (Wahle et al. 1972). This in vitro data indicates that the metabolic activity of the rumen mucosa and jejunal mucosa are quite comparable, although Wahle et al. (1972) expressed concern in regard to the width of their jejunal slices and diffusibility of oxygen into the interior of the tissue slice.

Webster (1980) suggested that the metabolic activity of the portal-drained tissues accounted for 0.20 of total metabolic heat generated in a sheep on a maintenance diet. This relationship seems to remain stable since heat production in the gut of starved sheep was measured at 62 kJ

kg $BW^{-0.75}$ day^{-1} which is 0.23 of total fasting heat production (Webster et al. 1975). Heat production in the gut tissues of sheep fed at two times maintenance was estimated at 0.23 of total heat production of the animal (Webster 1980). These results indicate that respiratory activity of the gut tissues varies little in relation to total heat production over a wide range of intakes. In addition, the gut tissues utilize a significant portion of total O_2 consumption and the metabolic heat generated by the gut tissues increases substantially with increasing intake.

Metabolic activity in relation to protein synthesis

The high metabolic activity of gut tissues may be a reflection of a high rate of protein turnover. Webster (1981) surmised that heat production and blood flow to a specific organ may be closely related to the amount of protein synthesis (and resynthesis) occurring in that tissue. Incorporation of labelled tyrosine into protein of certain body tissues, carried out in rats and cattle indicate that synthesis of protein in gut wall tissues accounts for about 0.4 of whole body protein synthesis and one-half of the oxygen consumed in these tissues (Webster 1980). In sixteen week old lambs, protein synthesis in the gut wall tissues is 0.26 of the total rate of protein synthesis in all of the major organs (Combe et al. 1979). The rate of protein synthesized in all of the gut wall tissues was estimated at 56.5 g day^{-1} and the rate of

synthesis within the walls of the stomach (reticulorumen, omasum, abomasum) was 31.5 g day $^{-1}$ or 0.56 of total gut tissue synthesis. It is attractive to conclude from the study of Combe et al. (1979) that protein synthesis in the walls of the ruminant forestomach accounts for over one-half of total gut tissue synthesis, however, it should be noted that Combe et al. (1979) included the abomasum in their assessment of protein synthesis in the stomach tissues. In lambs of similar age and weight, Davis et al. (1981) reported a rumen wall protein fractional synthesis rate of 0.79 per day. In simpler terms, the equivalent of 79% of the rumen wall protein was synthesized daily, which is an impressively high rate. It should be noted that the estimate of 0.79 was the upper estimate reported by Davis et al. (1981), calculated under the assumption that the specific activity of free leucine in the tissues could be equated with the specific activity of leucyl tRNA. As Davis et al. (1981) mentioned, this assumption may be uncertain and hence the upper estimate of 0.79 must be regarded with caution. Nevertheless, the estimate obtained for rumen wall was higher than those measured for liver, skeletal and cardiac muscle and skin (Davis et al. 1981).

It follows from the above discussion, that the high demand for oxygen in the gut tissues may result from the rapid rate of protein synthesis. Webster (1980) referred to the hyperplasia of the ruminal epithelium in response to increased feed intake (see previous section) as evidence for

this theory. A doubling of food intake resulted in a 67% increase in O_2 consumption by portal-drained viscera. Webster (1980) concluded from the data of Fell and Weekes (1975) that doubling of food intake would result in a 67% increase in the tissue mass of the rumen epithelium. Although the rumen epithelium is only one of the major portal-drained visceral tissues, the increase in heat production in the gut tissues seems to accompany the increase of tissue mass.

Clearly a larger gut tissue mass would require a corresponding increase in metabolism for maintenance, but there are certain other features to consider. An increasing epithelial tissue mass may simply reflect a decreased degradation rate of cells, in which case there need not be a corresponding increase in metabolic activity. However, accelerated gut tissue turnover would require increased metabolism in order to support a higher rate of mitotic division. Abrasive feedstuffs contributing to epithelial sloughing might be expected to result in increased mitosis in the gut epithelia. Measurement of [6-^{3}H] thymidine incorporation into DNA indicates that more fibrous feedstuffs may induce greater rates of rumen epithelial cell synthesis, which may be an indication of higher ruminal epithelium turnover rates (Rowe and James 1982). Cell production in the rumen epithelium and the small intestine accounted for 3 and 8%, respectively, of total body cell production (Rowe and James 1982). In addition, Sakata and

Tamate (1978,1979) provided evidence to indicate that the VFA may induce the proliferation of forestomach epithelial cells, especially if the levels of VFA increase transiently and periodically during the day.

The increase in O_2 consumption in the gut tissues in response to increased levels of feeding, as measured by Webster et al. (1975), may also be partially explained by utilization of O_2 within the lumen of the gut. Cheng et al. (1981) discussed the possibility that facultative anaerobes at the surface of the gut epithelium may utilize the O_2 which diffuses through the epithelium. As these authors suggested, this may be of quantitative importance in the reticulorumen where a substantial population of these microbes were reported to exist in association with the epithelium. It is conceivable that diffusion of O_2 through the epithelium may be accelerated by a higher O_2 utilization capacity by the microbes when there is an increased availability of substrates as a result of increased feed intake. It is also conceivable that increased food intake may stimulate subepithelial blood flow which would make more O_2 available for diffusion.

Despite all of this speculation, little is known of the total requirement for O_2 and the rates of protein synthesis in the gut as a whole, and even less is known about the specific tissues of the gut. It appears from the microsphere blood flow measurements (Hales 1973a,b; Engelhardt and Hales 1977), that the forestomach epithelium accounts for 0.2 to

0.3 of total blood flow to gut epithelia. Combe et al. (1979) found that protein synthesis rates in the stomach tissues accounted for a little over one-half of total protein synthesis in the gut wall tissues. Extrapolation of in vitro measurements of respiration rate indicates the rumen epithelium of a 25 kg sheep could utilize 5.7 liters of O_2 daily (calculated from in vitro respiration rate of 5 μl O_2 (mg dry weight)$^{-1}$ h^{-1} (Gieseke et al. 1979); rumen epithelial wet weight of 216 g for a 25 kg sheep on a maintenance diet (Engelhardt and Hales 1977); ovine ruminal epithelial dry matter content of 22% (Fell et al. 1972)). If one assumes that the energy equivalent of O_2 consumption is 20.4 kJ liter^{-1} (McLean 1972), the heat generated by rumen epithelium at the in vitro rate of respiration would be 116 kJ day^{-1} or 10.4 kJ kg BW$^{-0.75}$ day^{-1} , which is about 0.12 of the 90 kJ kg BW$^{-0.75}$ day^{-1} measured by Webster (1980) as the heat produced by portal-drained visceral tissues of a sheep on a maintenance diet. However, Mathison (1972) reported in vivo rates of O_2 consumption of 14 - 70 ml (kg rumen wall)$^{-1}$ min^{-1} in whethers on various diets, which translates into 22 - 110 kJ kg BW$^{-0.75}$ day^{-1} for a 40 kg sheep, a substantially larger proportion of the total portal-drained visceral heat production reported by Webster (1980). In view of these estimates it is difficult to appraise the contribution of the forestomachs to the consumption of O_2. Mathison (1972) did measure total O_2 consumption in 3 of the animals and rumen O_2 consumption accounted for 16, 5 and 24% of total

whole animal consumption.

Enzymatic activity

Occasionally, metabolic activity of a specific tissue can be assessed by the activity of certain enzymes. However, this method of appraising metabolic activity can be misleading since different tissues have differing functions. The presence of various metabolic pathways previously discovered in other tissues were in evidence in the epithelium of the ruminant forestomachs (Pennington 1952,1954; Pennington and Sutherland 1956a,b; Hird and Symons 1959,1961). Histochemical studies on bovine ruminal epithelium in calves and adults confirmed the operation of the pentose phosphate cycle, the Krebs cycle, glycolytic and ketogenic pathways by the identification of 10 intracellular dehydrogenases operating in these metabolic pathways (deLahunta 1965). deLahunta (1965) noted that the stratum corneum contained no dehydrogenase activity but lower layers of the epithelium all exhibited substantial staining with no apparent localization of any of the dehydrogenase enzymes. Supportive evidence for glycolysis and pentose phosphate cycle operation have also been provided by significant activities of glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) in bovine forestomach epithelium (Whanger and Church 1970). Rates of glucose conversion to pyruvate and lactate obtained by in vitro incubation of ruminal epithelium show active

rates of glycolysis (Weekes 1974; Emmanuel 1981b).

Various workers have identified a wide spectrum of enzymes involved in amino acid metabolism in forestomach epithelium. Since the epithelial tissue of the ruminant forestomach exhibits only a very minimal urea cycle capacity (Emmanuel 1980a) and low individual activities of urea-cycle enzymes (Chalupa et al. 1970; Whanger and Church 1970; Emmanuel 1980a), the presence of enzymes of amino acid metabolism is thought to provide a vehicle for ammonia anabolism and detoxification in the forestomach mucosa. NAD- and/or NADP- dependant glutamate dehydrogenase (EC 1.4.1.2 and EC 1.4.1.4) has been detected in the epithelium of the bovine, ovine and caprine rumen (Hoshino et al. 1966; Ide 1969; Chalupa et al. 1970; Whanger and Church 1970; Watson and Lindsay 1972; Weekes 1972; Nocek et al. 1980) but the activity of this enzyme is usually many fold less than the activity observed in liver tissue when expressed on a per wet weight basis or on a per mg protein basis (Whanger and Church 1970; Watson and Lindsay 1972; Salem et al. 1973).

Evidence of rumen mucosal glutamine synthetase (EC 6.3.1.2) activity is less convincing than that of glutamate dehydrogenase. Activity of glutamine synthetase comparable to liver activity of the enzyme has been observed in young bovine rumen mucosa (Salem et al. 1973), while Chalupa et al. (1970) reported only trace and variable amounts in the rumen mucosa of sheep.

Various other enzymes of amino acid metabolism found in rumen epithelium include glutamate-oxaloacetate transaminase (EC 2.6.1.1) and glutamate-pyruvate transaminase (EC 2.6.1.2) (Chalupa et al. 1970; Whanger and Church 1970; Weekes 1972,1974; Nocek et al. 1980). Whanger and Church (1970) claimed to detect transamination of the amino acids tryptophan, tyrosine and phenylalamine in ruminal epithelium of cattle.

The observations on enzymes involved in nitrogen metabolism are corroboratetd by tracer studies on the fate of ammonia and urea N in the forestomach wall. These studies have indicated that the forestomach epithleium is a potential site of N fixation into amino acids and other nitrogenous compounds. Mathison and Milligan (1971) suggested that this tissue is involved in the detoxification of ammonia and the interorgan movement and transfer of nitrogen. Studies performed with ^{15}N-urea indicate that the forestomach epithelium has a significant capacity to sequester ammonia N in organic forms for subsequent synthesis in this tissue or transfer of N back into the blood or the lumen (Havassy et al. 1974). Boila and Milligan (1980b) found a net release of amino acids from rumen papillae incubated in vitro with NH_4Cl and several sources of carbon. Rumen wall capacity for production or utilization of amino acids has also been reported from in vitro experiments (Leng et al. 1980), thus the presence of the enzymes reported above plus the other supportive evidence

are good indications of substantial coupling of ammonia N with carbon skeletons in the forestomach mucosa.

The enzymes involved in the initial metabolic steps of VFA metabolism have been closely evaluated in several studies carried out on both bovine and ovine ruminal epithelium. These enzymes are the short chain acyl-CoA synthetases (EC 6.2.1-) which catalyze the formation of the coenzyme A ester of acetate, propionate or butyrate. An accumulation of research has indicated that several acyl CoA synthetases exist in animal tissues, each with varying substrate specificities and other kinetic characteristics and it is presently thought that these varying properties may be involved in the regulation of VFA metabolism (see Groot et al. 1976 for a review). In vitro rates of activation of the VFA are consistently in the order of butyrate>propionate>acetate in homogenates or more purified preparations of ruminal epithelium when only one of the VFA substrates is present in the incubation (Cook et al. 1969; Ash and Baird 1973; Scaife and Tichivangana 1980). The inclusion of two or more VFA in the same incubation illustrates the effects of competition on the activation of any specific VFA to its respective acyl CoA. Ash and Baird (1973) working with bovine rumen epithelium and Scaife and Tichivangana (1980) with ovine epithelium, both observed that the rate of activation of propionate in rumen epithelium homogenates was severely curtailed by the presence of butyrate. Acetate activation rate was slightly

inhibited by the presence of propionate or butyrate. Ash and Baird (1973) concluded that the activation of VFA in rumen epithelium was regulated to favour metabolism of butyrate in this tissue. Investigation into acyl CoA synthetase kinetic properties allowed Scaife and Tichivangana (1980) to propose that this autoregulation is accomplished by the existance of two short-chain acyl-CoA synthetases in ovine rumen epithelium; one that is nonspecific for acetate, propionate, and butyrate and one which accepts only butyrate as the VFA substrate. The activation capacity for butyrate is larger and less susceptible to inhibition than activation of the other two VFA.

An additional unique aspect of the forestomach mucosa is the relatively continual production of ketone bodies (Pennington 1952; Pennington and Sutherland 1956a; Hird and Symons 1959;1961; Ramsey and Davis 1965; Roe et al. 1966; Weigand et al. 1972b; Baird et al. 1975; Goosen 1976; Giesecke et al. 1979; Emmanuel 1980b). Variations in production rate and relative output of acetoacetate and 3-hydroxybutyrate have prompted several investigations into enzymes and pathways of ketogenesis. Bush and Milligan (1971b) reported significant activity of three separate enzymatic reactions for conversion of acetoacetyl CoA to acetoacetate in crude extracts of bovine rumen epithelium while Emmanuel et al. (1982) proposed two main routes of 3-hydroxybutyrate synthesis from butyrate, one of which involved acetoacetyl CoA and acetoacetate as intermediates

and another which did not involve these intermediates.

Examination of enzymes present in forestomach epithelium and other ruminant tissues allows interesting comparisons to be made between tissues. Rumen epithelium often exhibits greater activity of 3-hydroxybutyrate dehydrogenase than the ruminant liver, either on a per wet weight of tissue basis (Watson and Lindsay 1972; Chandrasena et al. 1979) or on a per mg of protein basis (Chandrasena et al. 1979). In addition, about 0.8 of the 3-hydroxybutyrate dehydrogenase activity in the liver cell is found in the cytoplasm while the activity in rumen epithelial cells is distributed evenly between the cytoplasm and the mitochondria (Koundakjian and Snoswell 1970; Watson and Lindsay 1972).

Ash and Baird (1973) pointed out that acyl CoA synthetase activity was primarily limited to the particulate fraction of liver while the activity in rumen epithelium was evenly distributed between cytoplasmic and particulate fractions. Discussion above alluded to the studies of Scaife and Tichivangana (1980), which indicated the existence of a specific butyryl CoA synthetase in ruminal epithelium. Ricks and Cook (1981) have proposed that a specialized acyl CoA synthetase also exists in bovine liver mitochondria but that this enzyme exhibits a specificity for propionate. Ricks and Cook (1981) also isolated another mitochondrial acyl CoA synthetase, but this fraction displayed activity for several VFA including butyrate and valerate. The differences in acyl

CoA synthetases reflect the tissue preferences for substrate; the rumen epithelium utilizes butyrate to a larger extent than any other VFA (Pennington 1952; Stevens 1970; Weigand et al. 1975) and in most physiological conditions, propionate is almost completely removed from the portal venous system during its passage through the liver (Bergman 1975; Elliot 1980).

The activity of NADP-malate dehydrogenase or malic enzyme (EC 1.1.1.40) in rumen epithelium is much greater than in ruminant liver (Young et al. 1969; Whanger and Church 1970), and is comparable to the activity of malic enzyme in small intestinal mucosa (Whanger and Church 1970). The combination of the presence of malic enzyme activity and absence of phosphoenolpyruvate carboxykinase (EC 4.1.1.32) indicates participation of malic enzyme in conversion of propionate to pyruvate and lactate which has been reported in both in vitro and in vivo studies (Pennington and Sutherland 1956b, Leng et al. 1967; Weigand et al. 1972a; Weekes 1972).

B. Metabolism of volatile fatty acids

Acetate metabolism

It was noted previously that acetate is absorbed from the rumen less readily than propionate or butyrate. However, the in vitro or in vivo rates of transport of acetate across the rumen wall are consistently larger than for other VFA,

especially when the VFA are present in physiological concentration and proportion (Stevens 1970; Weigand et al. 1972b).

Acetate metabolism in gut tissue is more difficult to study in vivo than either propionate or butyrate because of the substantial endogenous production of acetate in body tissues in both the fed and the fasted states (Annison and Armstrong 1970; Bergman 1975; Pethwick et al. 1981). Consequently, much of the knowledge accumulated on acetate metabolism in gut tissues has been obtained from in vitro results.

During incubation of small pieces of ovine or bovine rumen epithelium there is net utilization of acetate and production of CO_2 and ketone bodies from the acetate (Pennington 1952,1954; Pennington and Sutherland 1956a). However, the rate of disappearance of VFA from the incubation was usually butyrate>propionate>acetate (Pennington 1952; Weigand et al. 1975) and the rate of ketone body synthesis from acetate was far less than the rate from butyrate (Pennington 1952,1954). Apparently, acetate was the least preferred VFA substrate for rumen epithelium and this was supported by the fact that acetate, in contrast to butyrate, did not induce an increase in tissue respiration rate (Pennington 1954). These observations were subsequently confirmed using isolated sheets of ruminal epithelium (Stevens and Stettler 1966a,1966b). In most cases, 0.4 - 0.6 of the acetate which

disappeared from the lumen solution appeared as acetate on the serosal side of the tissue and 0.11 - 0.17 of the acetate appeared as ketone bodies on either side of the tissue. The remainder of acetate which disappeared from the lumen solution was unaccounted for (Stevens and Stettler 1966a). In the absence of O_2, acetate metabolism to ketone bodies is abolished (Stevens and Stettler 1966a). There is no evidence to indicate that acetate is converted to any other VFA in rumen epithelium (Steven and Stettler 1966b).

Stevens (1970) concluded from in vitro experiments that 0.45 of absorbed acetate undergoes metabolism by the rumen epithelium. Conversion of acetate to ketone bodies could account for about one third of the 0.45, but the fate of the remaining acetate carbon was not discussed. Bergman and Wolff (1971) estimated from their in vivo work that 0.30 of acetate produced in the reticulorumen of the sheep does not appear as acetate in the portal blood, therefore it must be metabolized in the epithelium. Figure III.1 illustrates the utilization of both arterial and ruminal sources of acetate as derived from the data of Bergman and Wolff (1971). Indications of CO_2 production in the portal-drained viscera from arterial and ruminal sources of acetate are imprecise because of possible diffusion of CO_2 into the lumen. However, Pethwick et al. (1981) estimated that about 0.75 of the acetate utilized in the portal-drained tissues was directly oxidized to CO_2. Although their methods of measurement severely limit the conclusions, this estimate

does indicate substantial oxidation of acetate in the portal-drained viscera.

The activation of acetate, catalyzed by an acyl CoA synthetase, may well be the major site of regulation of acetate metabolism in forestomach epithelium (Cook et al. 1969; Ash and Baird 1973; Scaife and Tichivangana 1980). The major fate of acetate metabolized in the gut tissues appears to be its complete oxidation however, the quantitatively precise role of acetate in energy metabolism of the forestomach epithelium has not been established. The fact that, of the VFA, acetate is the least readily metabolized but yet the most abundant, could suggest a significant contribution to the overall energy metabolism in the forestomach epithelium.

Propionate metabolism

Although propionyl CoA synthetase activity in forestomach epithelium can be severely inhibited by the presence of butyrate (Ash and Baird 1973; Scaife and Tichivangana 1980), several studies have indicated substantial metabolism of propionate. Pennington (1952) observed that the rate of disappearance of propionate from the medium during incubation of small pieces of rumen epithelium increased when the oxygenating atmosphere contained a small percentage of CO_2. Unlike acetate and butyrate, propionate uptake did not give rise to formation of ketone bodies (Pennington 1952; Stevens and Stettler

1966a; Weigand et al. 1975). Propionate stimulated respiration of the rumen epithelium only in the presence of CO_2 (Pennington 1954) and later it was suggested that the initial metabolism of propionate involved carboxylation (Pennington and Sutherland 1956b). In addition, the conversion of propionate to CO_2 and lactate indicated that the rumen epithelium metabolized propionate in a manner similar to other animal tissues (Pennington and Sutherland 1956b). Stevens (1970) estimated that 0.65 of the propionate taken up by isolated sheets of bovine rumen epithelium was metabolized. Bergman and Wolff (1971) concluded that metabolism of propionate during absorption accounted for 0.5 of the amount absorbed since half of the propionate produced in the reticulorumen of the sheep did not appear as propionate in the portal blood.

Several studies on propionate metabolism in the forestomach epithelium indicate extensive conversion to lactate. Leng et al. (1967) concluded from propionate label incorporation into glucose that up to 0.7 of the propionate ultimately converted to glucose is first metabolized to lactate. The authors claimed that most lactate production from propionate ocurred in the rumen epithelium. If one considers that about 0.32 of the propionate produced in the rumen or 0.5 of the propionate absorbed into the portal venous system is converted to glucose in the sheep (Leng et al. 1967; Bergman et al. 1966), lactate production in the rumen epithelium could account for about 0.20 of propionate

production in the reticulorumen. On the basis of the results of Leng et al. (1967), Young et al. (1969) suggested that propionate metabolism to lactate in rumen epithelium was a means of conserving 3-carbon units.

However, net production rates of pyruvate and lactate from bovine and ovine papillae incubated with a variety of substrates indicated that propionate conversion to lactate (and pyruvate) was relatively insensitive to control by other substrates (Weekes 1972; Weekes 1974). Weekes (1972) stated that "this pathway provides a means of generating extramitochondrial NADPH required for synthetic processes within the mucosal cell". Although the portal-drained viscera are a major site of lactate formation (Annison et al. 1963a; Weekes and Webster 1975), it appears that the majority of lactate production in the portal-drained viscera is a result of glycolysis and not from propionate (Weigand et al. 1972a; Weekes and Webster 1975). In a study using [2-^{14}C] propionate, Weigand et al. (1972a) concluded that only 2.3% of absorbed propionate is converted to lactate in gastrointestinal tissues. In addition, propionate and lactate accounted for all of the radioactivity entering the portal venous drainage. If extensive propionate metabolism was occurring in the forestomach epithelium, the products certainly did not appear in the portal venous blood. The opinion of the authors was that very little of the absorbed propionate is metabolized by the reticulorumen of 250 kg calves in vivo.

Alternatively, Weekes and Webster (1975) observed a substantial discrepancy between the propionate absorbed from the rumen and propionate appearing in the portal venous blood which they attributed to extensive metabolism of propionate. However, lactate was not a major product of propionate metabolism in the rumen epithelium in their study.

Because there is evidence that propionate is metabolized during absorption, and lactate is not the major product, the question remains - what are the pathways of propionate metabolism in the rumen epithelium? In vitro results indicate that propionate can be oxidized to CO_2 in rumen epithelium (Pennington and Sutherland 1956b; Weigand et al. 1967). However, Goosen (1976) reported that only 2.1% of radioactivity from [2-^{14}C] propionate was isolated in CO_2 from incubated pieces of rumen epithelium. Propionate may also provide intermediates for the metabolism and detoxification of ammonia (Nocek et al. 1980) and synthesis of new cells. This may be the best explanation of propionate disappearance available considering the observation of Weigand et al. (1972a) (that very little radioactivity from ^{14}C-propionate was located in portal blood propionate metabolites), and the high cell turnover discussed in the previous section.

Butyrate and ketone body metabolism

Butyrate has been the most extensively studied VFA in forestomach epithelial metabolism, probably because it is the preferred VFA substrate in the tissue (Pennington 1954; Stevens and Stettler 1966a; Weigand et al. 1972b). In addition, in the fed state, the forestomach epithelium contributes over one-half of the total release of ketone bodies into the blood with butyrate being the major precursor of ketone bodies in the tissue (Baird 1981). The ketogenic role of forestomach mucosa may even continue in the fasted state, with ketone bodies being generated from long-chain fatty acids (Jackson et al. 1964; Hird et al. 1966; Cook et al. 1968).

Butyrate is a more effective ketogenic substrate than acetate when these two VFA are incubated separately with pieces of reticulum, rumen and omasum epithelium (Pennington 1952,1954). This is also illustrated by the fact that butyrate stimulates tissue O_2 uptake to a much larger extent than acetate (Pennington 1954; Goosen 1976). Anaerobic conditions result in a complete inhibition of formation of ketone bodies from butyrate or acetate (Hird and Weidemann 1964; Stevens and Stettler 1966a). Goosen (1976) found that ketone body production from bovine rumen epithelium incubated in 10 mM butyrate accounted for two-thirds of the total O_2 uptake of the tissue. In studies carried out on isolated sheets of rumen epithelium, butyrate transport to the serosal side of the tissue is almost always less than

0.1 of the butyrate which disappeared from the lumen solution (Stevens and Stettler 1966a). This prompted Stevens (1970) to conclude that metabolism in the rumen wall accounts for 0.85 of butyrate leaving the lumen. Butyrate conversion to ketone bodies by isolated sheets of rumen epithelium usually falls in the range of 0.5 - 0.7 of butyrate disappearance from the lumen bath (Hird and Weidemann 1964; Stevens and Stettler 1966a), and ketone body formation from incubated bovine rumen papillae accounts for 0.9 of the butyrate uptake (Weigand et al. 1975). In vivo evidence suggests that 0.78 - 0.94 of blood 3-hydroxybutyrate in fed sheep arises from rumen butyrate (Leng and West 1969). The extent of butyrate conversion to ketone bodies in vivo is less clear. Leng and West (1969) reported that only 0.15 of butyrate production in the sheep rumen was converted to ketone bodies. Weigand et al. (1972b) claimed that an average of 0.49 of butyrate was metabolized to ketone bodies during absorption in 250 kg calves.

Net portal appearance of butyrate and ketone bodies provides a good indication of substantial butyrate metabolism during absorption. The rates of butyrate entry into the portal venous blood or ruminal venous blood are very low in comparison with rumen concentrations of butyrate (Annison et al. 1957; Ramsey and Davis 1965; Bergman and Wolff 1971). In comparison with estimated rumen production rates of butyrate, portal appearance of butyrate indicates that 0.90 is metabolized during absorption (Bergman 1975).

The pathways of ketone body production in rumen epithelium have been the object of much investigation. Isotopic tracer studies gave indications that the majority of butyrate is incorporated into ketone bodies as a four-carbon unit rather than being broken down to acetyl units and subsequently synthesized into ketone bodies (Hird and Symons 1959,1961; Annison et al. 1963b; Ramsey and Davis 1965; Goosen 1976). Incorporation of label from acetate, or other substrates, was very low in the presence of butyrate (Hird and Symons 1961) which also indicates butyrate as the preferred ketogenic substrate in forestomach epithelium.

The exact pathways of ketogenesis from butyrate are more undefined and indications are that overall redox potential or energy status within the cell or compartments of the cell may affect the relative production of 3-hydroxybutyrate and acetoacetate. The redistribution of label from carbon 1 to 3 and from carbon 2 to 4 in the conversion of butyrate to ketone bodies, and the preference exhibited for the oxidation of carbons 1 and 2 of butyrate, inferred the presence of a 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) pathway in rumen and omasum epithelium of the sheep (Hird and Symons 1961). The operation of this pathway was later confirmed by enzymatic activity of HMG-CoA synthase (EC 4.1.3.5) in bovine rumen, reticulum, and omasum epithelium (Baird et al. 1970; Bush and Milligan 1971b). In a comparison of HMG-CoA synthase activity with the activity of 3-oxoacid CoA transferase (EC 2.8.3.5) and acetoacetyl

CoA deacylase (EC 3.1.2.11), Bush and Milligan (1971b) found that the transferase accounted for 70% of the total capacity to convert acetoacetyl CoA to acetoacetate in rumen epithelium. As pointed out by the authors, the major contribution of this transferase to epithelial ketogenesis has implications on the energetics and control of ketone body formation in the forestomach epithelium .

Continued studies on ketogenesis, and particularly on 3-hydroxybutyrate synthesis, in rumen epithelium has indicated the possibility of a route of D(-)-3-hydroxybutyrate formation that completely circumvents reduction of acetoacetate by 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) (Emmanuel et al. 1982) (see Figure III.4). In separate measurements of D(-)-3-hydroxybutyrate production, the potential for production by route B was 0.60 the rate by route A in both ovine and bovine rumen epithelium (Emmanuel et al. 1982). This may indicate that the alternate route of ketogenesis may be quantitatively significant in vivo even though production rates of D(-)-3-hydroxybutyrate may be much lower in vivo than tissue homogenate production rates (Emmanuel et al. 1982).

The effects of additional substrates on in vitro ketogenesis from rumen epithelium serve to illustrate some aspects of ketone body formation in vivo. Net production of 3-hydroxybutyrate in the gut is 2.8 - 4.5 times the production of acetoacetate in fed, lactating or non-lactating dairy cows (Baird et al. 1975; Baird 1981) and

2.3 - 4.7 times in 250 kg calves (Weigand et al. 1972b). In vitro incubation of rumen epithelium with butyrate usually results in a lower 3-hydroxybutyrate/acetoacetate (+acetone) ratio than in vivo measurements (Hird and Symons 1959; Sutton et al. 1963b; Stevens and Stettler 1966a; Weigand et al 1975; Goosen 1976; Emmanuel 1980b). However, addition of glucose to the incubation medium causes a shift towards a greater reduction of ketone bodies, so that the 3-hydroxybutyrate/acetoacetate approaches in vivo values (Hird and Symons 1959; Goosen 1976; Giesecke et al. 1979). In many cases, the inclusion of glucose into an incubation medium already containing butyrate will stimulate an increase in O_2 uptake and an increase in 3-hydroxybutyrate production rate (Hird and Symons 1959; Goosen 1976; Giesecke et al. 1979; Stangassinger et al. 1979) which is contrary to the antiketogenic effect of glucose in ketotic animals. A number of workers have suggested that glucose stimulation of rumen epithelium ketogenesis from butyrate results from charges in the $NADH/NAD^+$ ratio in the epithelium and sparing of butyrate oxidation (Hird and Symons 1959; Goosen 1976; Stangassinger et al. 1979). Stangassinger et al. (1979) pointed out that glucose would stimulate pyruvate dehydrogenase in the mitochondria, thus stimulating intramitochondrial $NADH/NAD^+$. Since 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) activity is equally distributed in the soluble and particulate fractions of the rumen epithelial cell (Watson and Lindsay 1972) reduction of

acetoacetate to 3-hydroxybutyrate is likely to be much more prevalent in rumen epithelium than in liver, where 3-hydroxybutyrate dehydrogenase activity is located in the cytoplasm (Koundakjian and Snoswell 1970; Watson and Lindsay 1972). Stangassinger et al. (1979) also suggested that stimulation of pyruvate dehydrogenase by glucose would reduce need for oxidation of butyrate carbon to CO_2, due to increased supply of mitochondrial acetyl CoA.

Propionate exhibits affects on hepatic and rumen epithelial ketogenesis similar to those of glucose; it is antiketogenic in the liver but displays very little effect on ketone body production from butyrate in rumen epithelium (Bush et al. 1970). However, the mechanism of hepatic propionate antiketogenesis is somewhat different than glucose. Investigations by Bush and Milligan (1971a) revealed that the mechanism of hepatic antiketogenesis by propionate was due to inhibition of HMG-CoA synthetase by propionate and propionyl CoA. As referred to earlier in this chapter, since the HMG-CoA pathway is not the major route of ketogenesis in rumen epithelium, propionate does not induce inhibitory effects in rumen epithelium (Bush and Milligan 1971b).

Incubation of rumen epithelium with all three VFA does seem to result in a significant reduction in ketogenesis. Goosen (1976) reported that propionate was antiketogenic in rumen epithelium when the tissue was incubated with propionate, acetate and butyrate as opposed to incubation

with butyrate alone. The reduction may be related to the fact that propionate may severely curtail the metabolism of acetate in rumen epithelium (Pennington and Pfander 1957). Propionate or a metabolite of propionate may inhibit metabolism of acetate, perhaps via inhibition of acetate activation, which in turn requires more butyrate to be completely oxidized rather than being reserved for ketogenesis.

Oxidation of butyrate occurs via the breakdown of acetoacetyl CoA into two acetyl CoA units and subsequent entry of acetyl CoA into the tricarboxylic acid cycle. The significance of butyrate oxidation by forestomach epithelium appears to be quite small. Emmanuel (1980b) reported that slightly less than 0.2 of the total amount of butyrate metabolized in sheep or goat rumen epithelium was converted to CO_2. During incubation of bovine rumen or omasum epithelium with 1-^{14}C-butyrate, only 0.8 - 3.5% of the radioactivity added was found in CO_2 (Hird and Symons 1959; Goosen 1976) while 12.6% of 1-^{14}C-butyrate radioactivity appeared in acetoacetate (Goosen 1976). The extent of complete oxidation of substrates in forestomach epithelium seems to be quite minimal, and it remains to be discovered whether any substrate is substantially oxidized to CO_2 in rumen epithelium in vivo.

Metabolism of higher volatile fatty acids

The incubation of rumen papillae from young steers with iso-butyrate, n-valerate or iso-valerate resulted in a net uptake of each one of these minor VFA (Weigand et al. 1975). Weigand et al. (1975) reported that the net rates of uptake by the papillae were butyrate>>n-valerate,propionate>iso-butyrate>iso-valerate. Metabolism of n-valerate resulted in substantial production of lactate, but very little of iso-butyrate or iso-valerate uptake could be accounted for in either ketone bodies or lactate. The n-butyrate to iso-butyrate ratio of 7 in rumen fluid is often reduced to less than unity in ruminal venous blood (Ramsey and Davis 1965) which indicates that iso-butyrate is metabolized to a far lesser extent than n-butyrate. The small amounts of these higher VFA in forestomach contents and also in blood plasma make study of absorption and metabolism in gut tissues very difficult.

C. Metabolism of glucose

The characteristics of in vitro glucose metabolism by forestomach epithelium are as follows: glucose may, or may not, stimulate tissue respiration rates above endogenous rates of oxygen consumption (Pennington 1952; Hird and Symons 1959; Goosen 1976; Giesecke et al. 1979); ketogenesis from butyrate is often augmented by the addition of glucose (Goosen 1976; Giesecke et al. 1979; Stangassinger et al. 1979); lactate and pyruvate may be formed from glucose or

propionate but glucose plus propionate will result in a synergistic increase in lactate and pyruvate production (Weekes 1974); and glucose is relatively more preferred by rumen epithelium from animals which have not developed extensive fermentation (Giesecke et al. 1979).

The addition of glucose to an incubation medium raises the 3-hydroxybutryate to acetoacetate ratio to a level approximating the ratio of these ketone bodies entering the portal vein (Goosen 1976; Stangassinger et al. 1979; Weigand et al. 1972b). This may be an indication of in vivo glucose metabolism by forestomach epithelium.

The oxidation of glucose to CO_2 has been measured at 1.68 µmoles glucose (g wet weight)$^{-1}$ h^{-1} in incubated rumen epithelium from mature sheep (Emmanuel 1981a). Lactate formation from glucose was 4.15 µmoles lactate (g wet weight)$^{-1}$ h^{-1} under similar conditions (Emmanuel 1981a). It appears that glucose may spare the oxidation of other substrates and therefore may be an important metabolic substrate in vivo.

Although there is evidence of substantial glucose uptake and glycolysis to lactate in the portal-drained viscera of ruminants (Weigand et al. 1972a; Bergman 1975; Baird et al. 1975; Weekes and Webster 1975; Huntington et al. 1980), there is little information available on in vivo glucose utilization by forestomach epithelium. Broad et al. (1980) provided evidence of substantial glucose removal from the plasma by the rumen wall. By placing catheters into

ruminal veins of anaesthetized sheep, the authors measured arteriovenous differences of 54 mg dL^{-1} across the rumen wall, which in this case was equivalent to the extraction of 0.3 of the plasma glucose during passage through the vascular system of the rumen wall (arterial plasma levels of glucose were high in this study). Assuming that 0.9-0.95 of the rumen wall capillary blood flow passes through the subepithelium rather than through the musculature (Engelhardt and Hales 1977), virtually all of the glucose extracted would be available to the epithelium.

Based upon evidence of glucose utilization by epidermal cells, Fell and Weekes (1975) speculated that there is an epithelial cell glucose requirement for mitosis and the synthetic phase of development. The later stages of development, which entail transformation into an inert "horny" cell (Lavker and Matoltsy 1970), requires little input of substrate.

D. Amino acid metabolism

Webster (1980) speculated that protein turnover in the gut tissues may account for one half of the total energy expended in these tissues. If this estimation is accurate, the metabolism of amino acids in forestomach epithelium may be of great quantitative importance.

Venoarterial concentration differences observed in fed sheep and cattle indicate that most amino acids are added into the blood plasma by the portal drained viscera (Hume et

al. 1972; Wolff et al. 1972; Baird et al. 1975; Bergman and Heitmann 1978; Tagari and Bergman 1978). This is not an unexpected observation since a whole spectrum of amino acids are presumably available for absorption from the gut. However, discrepancies exist in respect to the amount of certain amino acids present in the digesta and the appearance of those amino acids in the portal venous ciculation (Hume et al. 1972; Tagari and Bergman 1978). This phenomenon is most notable for glutamate and glutamine, which are often utilized in the sheep gut tissues to a larger extent than they are released into the circulation (Hume et al. 1972; Wolff et al. 1972; Heitmann and Bergman 1978, 1981), and alanine and proline, which are released into the circulation in far greater proportions than are present in the digesta (Hume et al. 1972). Tagari and Bergman (1978) provided corroborating evidence for the utilization of glutamate and the generation of alanine in the intestinal tissue of sheep by observing little portal appearance of abomasally-infused glutamate but considerable portal appearance of abomasally-infused alanine. They concluded that both arterial and intestinal glutamate and alanine are metabolized; glutamate undergoes almost complete metabolism in the intestinal mucosa and alanine undergoes substantial metabolism, but is also generated from 3-carbon intermediates and amino nitrogen from degradation of other amino acids (Tagari and Bergman 1978). This accounts for the net disappearance of glutamate from the portal circulation,

and the net appearance of alanine into the portal circulation.

Owing to the close metabolic relationship with glutamate, the fate of glutamine seems to be much the same as glutamate. Extensive studies have been carried out on glutamine utilization in the small intestine (Windmueller 1982). In situ perfusions of isolated segments of intestine of fasted rats indicate that 30 - 35% of the total plasma glutamine is extracted during passage through this gut tissue (Windmueller 1982). This translates into a glutamine utilization rate which is similar to the utilization rate of glucose in the same preparations. In fed rats, the utilization rate of glutamine is doubled (Windmueller 1982). Over 50% of total glutamine carbon metabolized in an auto perfused segment of rat jejunum is completely oxidized to CO_2 (Windmueller 1982). The other metabolic products include lactate, citrate and other organic acids (malate, pyruvate, succinate, α-ketoglutarate), proline, citrulline, alanine and glucose (Windmueller 1982). Of the glutamine utilized by the jejunum, almost 100% of the nitrogen was recovered in the venous effluent, with ammonia, citrulline and alanine accounting for 90% of the total (Windmueller 1982).

On the basis of these observations, arterial and luminal glutamine appears to be a major respiratory fuel for the small intestine of the rat and it may also function as an important interorgan intermediate of nitrogen metabolism (Windmueller 1982).

Broad et al. (1980) also implicated glutamine as an important metabolite for nitrogen transfer into the rumen via the rumen wall. These workers measured arteriovenous differences in blood metabolites across the rumen wall of anaesthetized sheep and observed that about 11% of arterial plasma glutamine was extracted by the rumen wall. However, the apparent utilization of glucose by the rumen wall was many fold larger than that of glutamine.

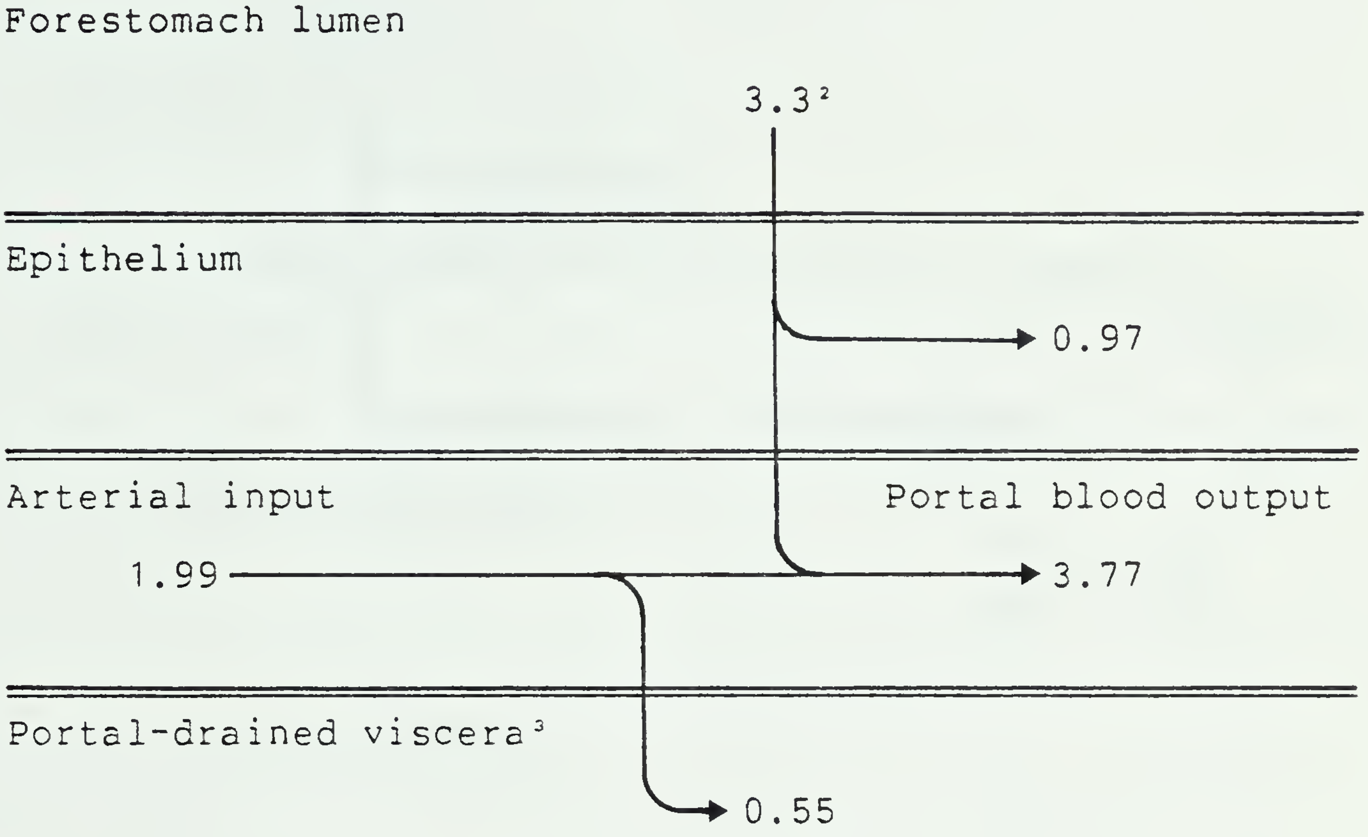
Nevertheless, the study of Broad et al. (1980) suggests extensive amino acid metabolism in forestomach epithleium. Although there have been reports of amino acid absorption from the forestomach (Cook et al. 1965; Liebholz 1971a,b), the quantitative importance of this source of amino acids is likely limited by the very low concentrations of free amino acids in the reticulorumen fluid. It is more likely that amino acid metabolism in the rumen wall involves arterial sources of metabolites and intermediates and products of microbial metabolism in the reticulorumen. Boila and Milligan (1980a,b) have studied the release of amino acids from rumen papillae incubated in the presence of various sources of carbon and nitrogen. The in vitro release of valine, threonine, isoleucine, proline, leucine, phenylalanine, lysine, tyrosine, methionine and arginine were unaffected by inclusion of any of the substrates studied (Boila and Milligan 1980b). The amino acids produced from the addition of substrates to the incubation medium included serine, glycine, alanine, aspartate plus asparagine

and glutamate plus glutamine (Boila and Milligan 1980b). These amino acids are represented in Figure III.5, which is a proposed scheme of amino acid metabolism in forestomach mucosa, based on the available information of enzymatic activities and in vitro observations of arteriovennous extraction/addition of metabolites in the portal-drained viscera or rumen wall.

The reader is referred to Boila and Milligan (1980a,b) for a more complete discussion of amino acid synthesis from the various substrates but it is relevant to point out a few observations. The only carbon substrate which stimulated a significant synthesis and release of total amino acids in the presence of NH_4Cl as a nitrogen source, was glyoxylate (Boila and Milligan 1980b). The main effects of glyoxylate were manifested upon serine and glycine, which is consistent with the metabolic scheme presented in Figure III.5. The carbon substrates glucose and propionate actually depressed the release of amino acids from the incubated papillae (Boila and Milligan 1980b). In the presence of carbon skeleton precursors, glutamate was often a better source of nitrogen than glutamine in the synthesis of amino acids (Boila and Milligan 1980b). This observation appears to be inconsistent with the evidence presented for the small intestine, where glutamine is utilized to a larger extent than glutamate. In a total summary of amino acid secretion from incubated rumen papilla, Boila (1977) concluded that the availability of carbon sources would be the critical

determinant of amino acid synthesis in vivo. Present knowledge suggests that the major anabolic carbon skeletons supplied to the forestomach epithelium include propionate, glucose, glutamate, and glutamine.

Figure III.1. Acetate utilization in portal-drained viscera of the sheep[1].


Forestomach lumen
3.3[2]
Epithelium
0.97
Arterial input
Portal blood output
1.99
3.77
Portal-drained viscera[3]
0.55

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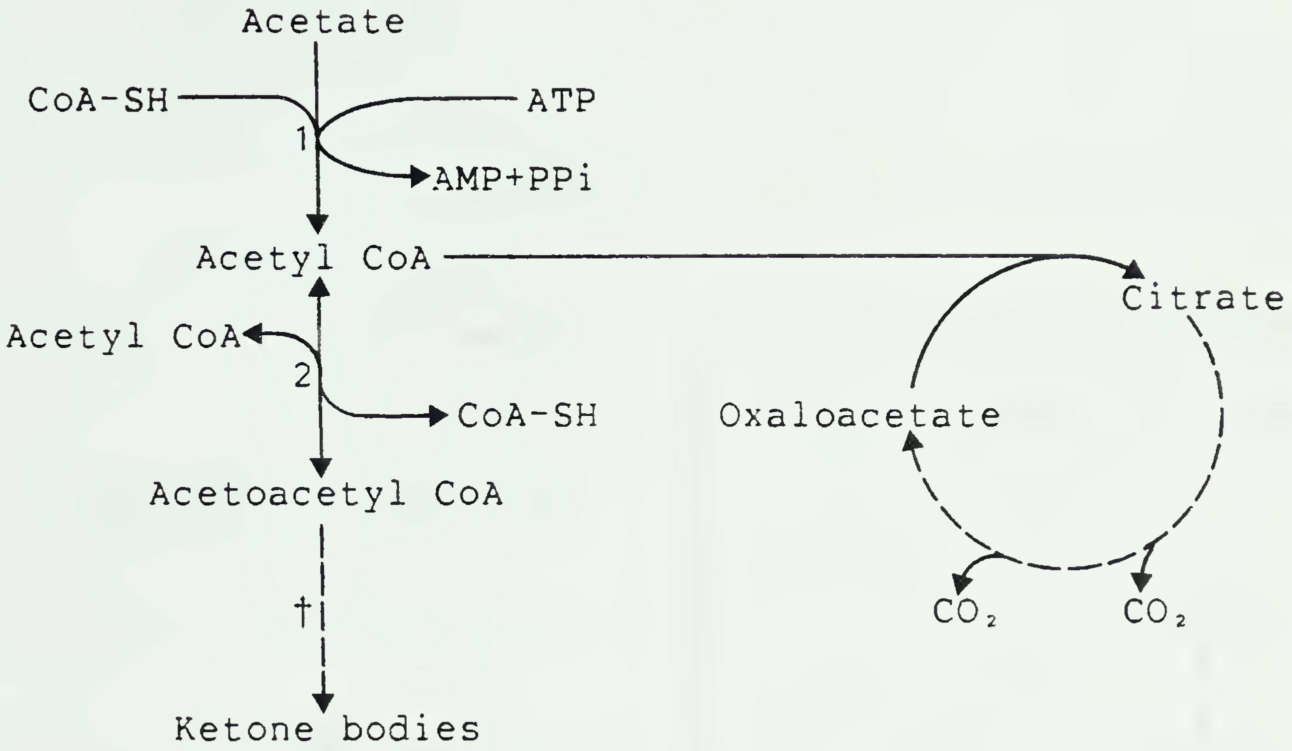
[1] acetate flux rates (moles day^{-1}) from Bergman and Wolff (1971).

[2] estimated reticulorumen production rate of acetate (Bergman et al. 1965; Leng and Leonard 1965).

[3] some of the arterial acetate metabolized in portal-drained viscera may be metabolized within the forestomach epithelium.

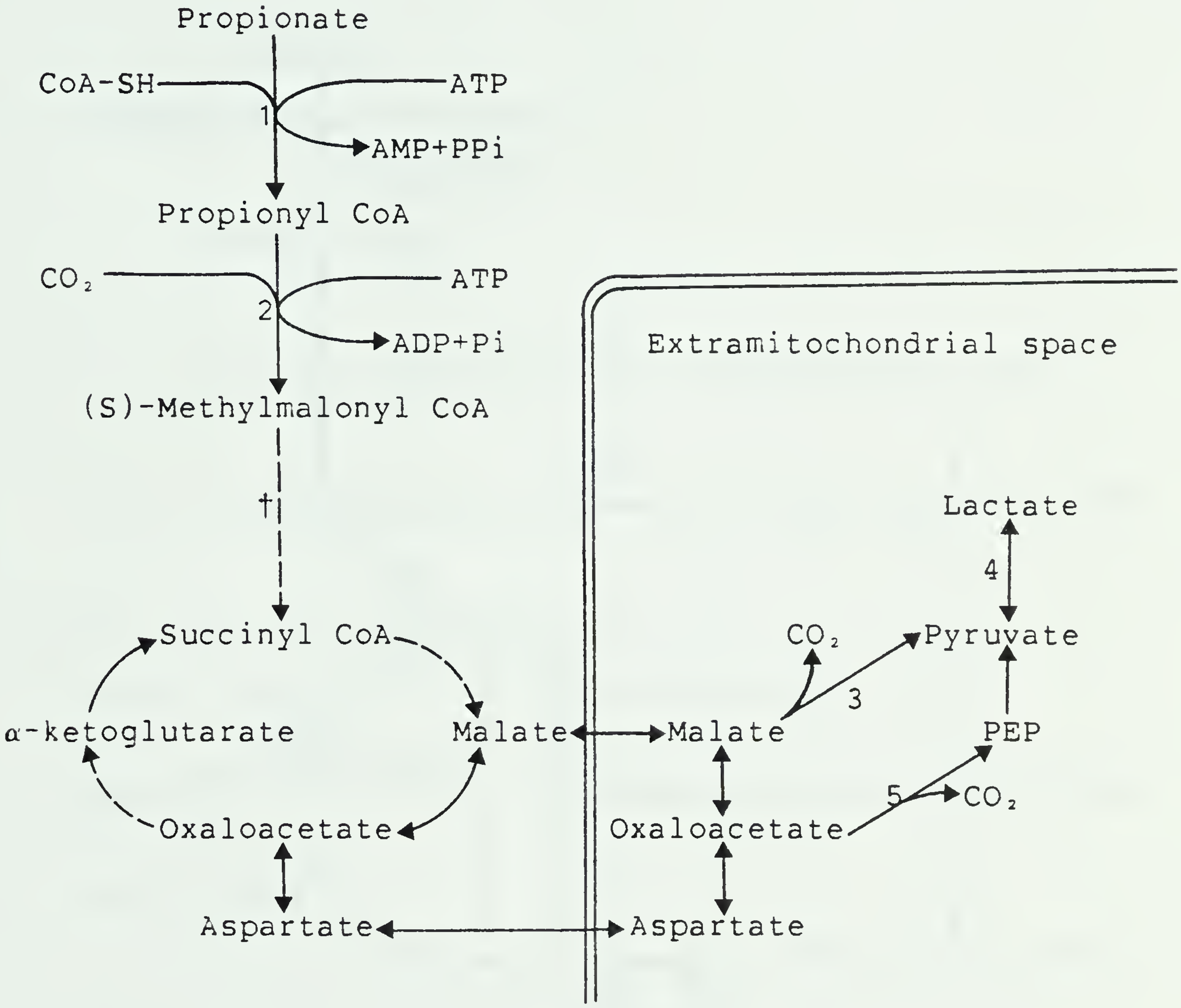
Figure III.2. Major pathways of acetate metabolism in rumen epithelium.


Acetate
CoA-SH
ATP
1
AMP+PPi
Acetyl CoA
Citrate
Acetyl CoA
2
CoA-SH
Oxaloacetate
Acetoacetyl CoA
†
CO₂
CO₂
Ketone bodies

1. Acyl CoA synthetase (EC 6.2.1-)
2. Acetyl CoA thiolase (EC 2.3.1.9)

† dashed lines signify pathways with more than one enzymatic step.

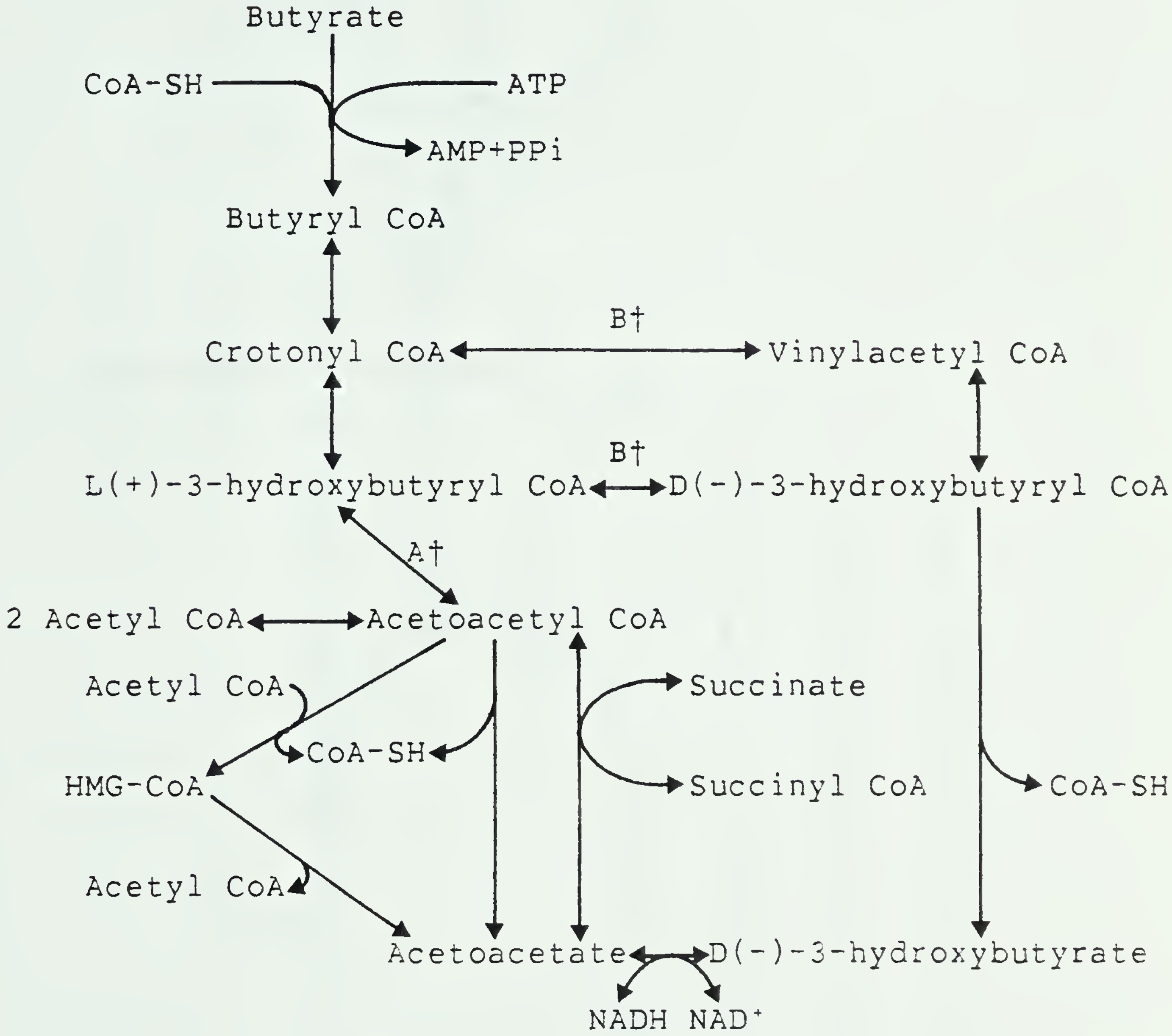
Figure III.3. Major pathways of propionate metabolism in rumen epithelium.


Propionate
CoA-SH
ATP
1
AMP+PPi
Propionyl CoA
CO_2
ATP
2
ADP+Pi
(S)-Methylmalonyl CoA
†
Succinyl CoA
α-ketoglutarate
Malate
Oxaloacetate
Aspartate
Extramitochondrial space
Lactate
4
CO_2
Pyruvate
3
Malate
PEP
5
CO_2
Oxaloacetate
Aspartate

1. Acyl CoA synthetase (EC 6.2.1-)
2. Propionyl CoA carboxylase (EC 6.4.1.3)
3. Malic enzyme (EC 1.1.1.40)
4. Lactate dehydrogenase (EC 1.1.1.27)
5. Phosphoenolpyruvate carboxykinase (EC 4.1.1.32)- limited activity in rumen epithelium.

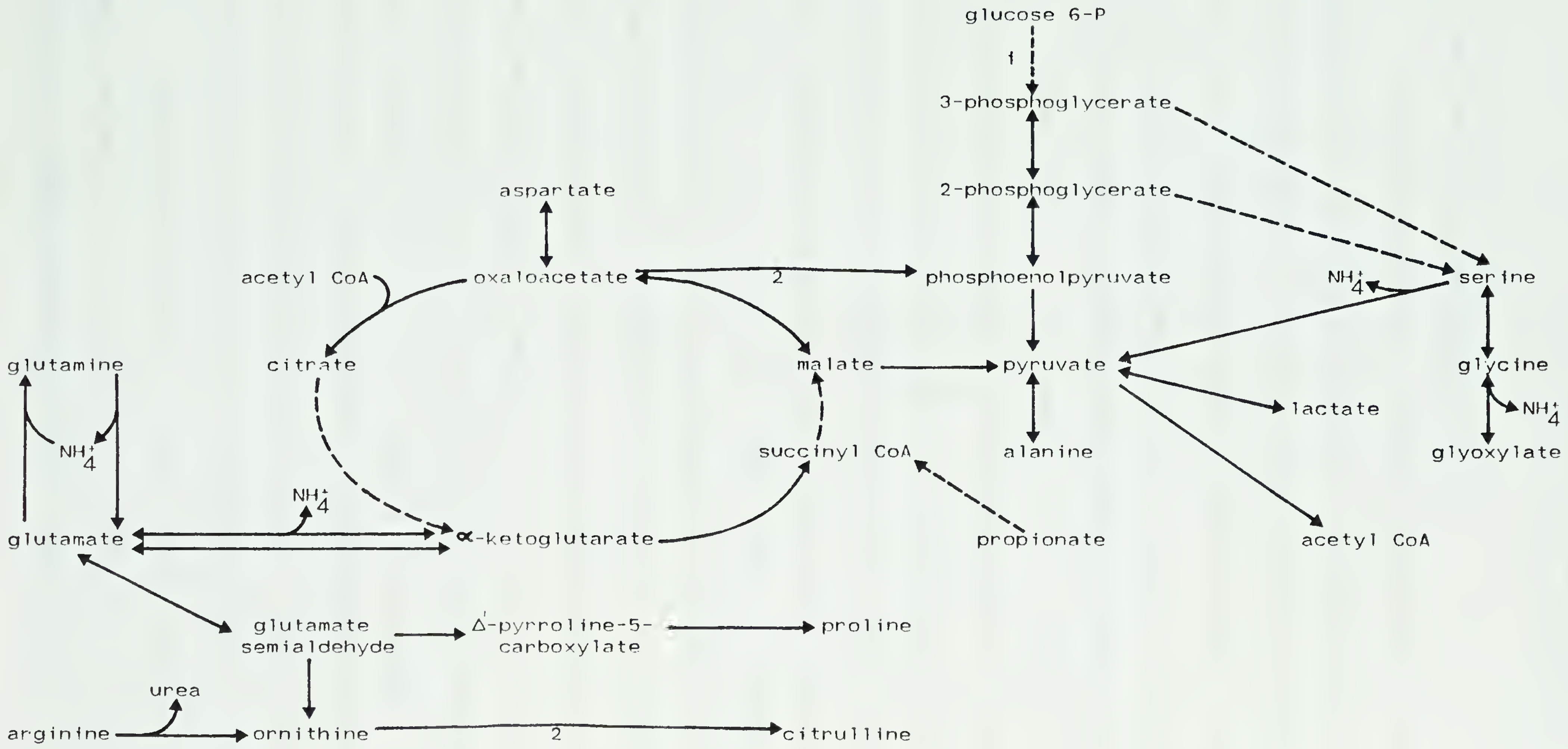
† a dashed line signifies a pathway with more than one enzymatic step.

Figure III.4. Major pathways of butyrate metabolism and ketone body formation in rumen epithelium (Emmanuel et al. 1982).


Butyrate
CoA-SH
ATP
AMP+PPi
Butyryl CoA
Crotonyl CoA
B†
Vinylacetyl CoA
L(+)-3-hydroxybutyryl CoA
B†
D(-)-3-hydroxybutyryl CoA
A†
2 Acetyl CoA
Acetoacetyl CoA
Acetyl CoA
CoA-SH
Succinate
HMG-CoA
Succinyl CoA
CoA-SH
Acetyl CoA
Acetoacetate
D(-)-3-hydroxybutyrate
NADH
NAD+

† A and B signify D(-)-3-hydroxybutyrate production by routes A and B respectively (Emmanuel et al. 1982).

Figure III.5. Proposed pathways of amino acid metabolism in ruminal epithelium.


glucose 6-P
1
3-phosphoglycerate
2-phosphoglycerate
aspartate
acetyl CoA
oxaloacetate
2
phosphoenolpyruvate
NH_4^+
serine
glutamine
citrate
malate
pyruvate
glycine
lactate
NH_4^+
NH_4^+
succinyl CoA
alanine
glyoxylate
NH_4^+
glutamate
α-ketoglutarate
propionate
acetyl CoA
glutamate semialdehyde
Δ'-pyrroline-5-carboxylate
proline
urea
arginine
ornithine
2
citrulline

1. dashed lines represent pathways with more than one enzymatic step.
2. enzymatic step with limited activity in rumen epithelium.

IV. MATERIALS AND METHODS

A. Method of respiration study

A physiologically valid assessment of metabolism and respiration of the forestomach epithelium requires conditions which are not excessively removed from those in vivo, and yet can be controlled in order to derive quantitative information. As a result, vascular perfusion of isolated areas of the forestomach epithelium was undertaken.

The laminae, or leaves, of the omasum are well suited for perfusion because of the anatomy of the vascular system. Several arteries descend into the leaves from the greater curvature of the omasum. The veins which return the blood to the surface of the organ are directly alongside descending arteries (Comline et al. 1968) and an adjacent vein can be clearly identified in a freshly collected leaf due to the remaining blood trapped in the major vessels of the leaf. This allows one to trace the coupled artery and vein within an isolated leaf. The point of entry of a descending artery and ascending vein can be quickly recognized by the naked eye, pinpointed with the aid of a low power dissecting microscope, and successfully catheterized by properly prepared and sized stainless steel capillary tubes or hypodermic needles with a minimum amount of practice.

Since each artery - vein couple supplies a relatively small area, it is possible to achieve a closed system with which to study metabolism and respiration. Preparation of an

area for study is accomplished by clamping the tissue in a specially developed plastic chamber (see Appendix I) in which an area 4.5 cm in diameter (diameter of inner O-ring) is isolated. Proper catheterization and mounting in the chamber allows perfusion of a circular area of tissue with total collection of effluent. In addition, the solutions bathing the lumen sides of the leaf can be controlled and are accessable via small ports entering each side of the lumen cavity of the chamber (see Appendix I).

B. Collection and transport of tissue

Bovine omasal tissue was obtained at a local slaughterhouse (Gainers Ltd.) from freshly killed animals. Time duration between killing and accessability to the omasum of the animal was approximately 12 min. Tissue from which results were reported in these studies were obtained from slaughter market steers and heifers (400 - 650 kg). Most of these animals were on a mixed grain: roughage diet as judged by the contents within the rumen and omasum. Age of the animals was unknown and thus age, physiological status and diet must be considered as major factors contributing to variability between tissue obtained from the different animals.

The omasum was separated from the rest of the gut tissue and subsequently sliced open in an approximate midline section across the greater curvature of the organ, parallel to the attachment of the leaves to the omasal wall

(convex edge; Church 1976). After completion of this incision, the omasum could be folded open and a leaf selected for transport back to the laboratory. Since success of catheterization was partially dependent on the size of the descending vessels, full-sized or three-quarter sized leaves were usually selected and cut free from the omasal wall.

The tissue was transported to the laboratory in 1 L of 75 mM THAM (tris(hydroxymethyl)aminomethane): 75 mM NaCl buffer pH 7.4 at 20±2 °C. In addition, 0.06 mg of nitroglycerin (Parke-Davis) was routinely included in the transport buffer since it was found to be effective in maintaining dilated and relatively rigid arteries.

C. Preparation of tissue for perfusion

Upon reaching the laboratory, the leaf was rinsed with 38 °C 0.15 M NaCl. Any attached contents were removed by quickly rinsing in warm water and then in 0.15 M NaCl. The tissue was then mounted under a dissecting scope in preparation for catheterization of a descending artery and its adjacent vein.

Catheterization

Whenever possible, the pair of vessels chosen for catheterizataion were closer to the distal (abomasal) end of the leaf, about 0.25 of the way along the convex edge of the leaf from the abomasal to the reticular end. The microscope

was centered above the point of entry and exit of the vessels and the tissue was teased open at this point to reveal a 1 cm section of exposed artery and vein (see Appendix I). The tissue could then be appropriately stretched and pinned to the mounting board in order to enable easier catheterization.

The arterial catheter consisted of polyvinyl chloride micro-bore tubing (0.254 mm I.D. X 0.762 mm O.D.; Cole Parmer) with a 2 cm segment of stainless steel capillary tubing (0.152 mm I.D. X 0.305 mm O.D.; Small Parts Inc.) inserted into the polyvinyl chloride tubing so that approximately 1.5 cm protruded from the end of the catheter. The stainless steel end of the catheter was carefully introduced into the artery and pushed in to a point where it was 0.5 cm beyond the exposed portion of the vessel[1]. When the arterial catheter was in place, the vessel was flushed with heparinized saline (37 °C) until effluent was observed flowing out of the adjacent vein. This also helped to pinpoint the exact position of the vein, which was subsequently catheterized with a stainless steel tube[1] (0.584 mm I.D. X 0.889 mm O.D.) prepared from a 20 G hypodermic needle. The stainless steel tube was connected to a 12 cm length of 0.508 mm I.D. X 1.524 mm O.D. polyvinyl chloride tubing for transport of the effluent.

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[1] The tips of the stainless steel tubes used for catheterization of both the artery and the vein were carefully ground and rounded to avoid vessel damage upon insertion.

When both catheters were inserted, they were secured to the tissue by a suture around both of the stainless steel tubes at a point where they were embedded in the unexposed vessels (see Appendix I). The tissue was then flushed with heparinized saline to establish flow into the tissue and out of the venous catheter. If flow could not be established, or if excessive leaking occurred, catheterization was attempted at another location.

Mounting the tissue in the perfusion chamber

Once the catheters were secured to the tissue, the tissue and catheters were carefully moved into position on the base of the perfusion chamber (see Appendix I). The catheter tubes were positioned in the groove running from the interior to the exterior of the chamber. When setting up the tissue in the chamber, the stainless steel tubes were situated so that only a few mm protruded into the lumen cavity and the catheterized vessels were centered down the middle of the chamber cavity. The tissue was stretched across the chamber cavity, and then the other half of the perfusion chamber was lowered onto the tissue. The tissue was carefully clamped in between the two halves of the perfusion chamber with special attention given to maintaining pressure in the vascular system while clamping the chamber tight. This was accomplished by continued flushing of the tissue with heparinized saline during the clamping procedure.

Duration between collection of tissue at the abattoir and completion of mounting was usually 40 - 50 min.

D. Tissue perfusion

Perfusion and chamber media

All of the solutions used to perfuse or to bathe the lumen sides of the omasal tissue were prepared fresh daily. Perfusate was equilibrated with carbogen (95% O_2:5% CO_2) at 38 °C for at least 2 h before use and adjusted to pH 7.2 at 38 °C after an equilibration period of at least 1 h. The Hepes Perfusion Buffer (HPB) (Table IV.1) was continuously equilibrated with carbogen for the duration of its use.

Basically, two solutions, similar to HPB (Table IV.1), were used in the lumen compartment of the perfusion chamber. The initial chamber solution was vigorously boiled for 5 min and then maintained at 38 °C until use. The pH of the initial lumen-bathing solution was not adjusted after boiling; pH at 38 °C was 7.16±0.021 and 7.19±0.007 (mean±SE) for Experiments I and II respectively.

In order to attain more physiological conditions, one of the chamber solutions was made anaerobic. The solution was maintained at 50 - 60 °C for about 30 min. then brought to a boil for 2-3 min. During all of this period, the solution was gassed with O_2-free N_2 , which was prepared from commercial N_2 by passage through a 350 °C tubular furnace filled with copper shavings. The copper turnings in

the furnace were reduced with H_2 for 1 h at 350 °C for every 1 h of use in order to maintain the efficacy of the furnace during experiments. After boiling, a few crystals of purified sodium dithionate ($Na_2S_2O_6 \cdot 2H_2O$) were added to remove the last traces of O_2. Addition of the sodium dithionate crystals resulted in an abrupt clearing of the solution due to the reduction of free O_2 and the resultant disappearance of oxidized resazurin colour. The solution was maintained at 50 °C and continuously gassed with O_2-free N_2 until immediately before use. Solution was withdrawn as needed, from the stock flask by a glass syringe and allowed to cool to 38 °C before use; pH at 38 °C was 7.16±0.014 and 7.18±0.007 (mean±SE) for Experiments I and II respectively.

Tissue perfusion

Upon mounting in the perfusion chamber, the lumen compartments were filled with initial solution and the arterial catheter was subsequently connected to the head of a positive displacement pump (Technicon Corporation). Perfusion commenced at the rate of 1 ml min^{-1}, with the perfusate being withdrawn from the reservoir of HPB solution (see Figure IV.1). The perfusion chamber was kept at 38 °C for the remainder of the experimental run. After a 2 min period, the venous effluent was directed into the sample chamber of a Clark-type polarographic O_2 electrode, via the access groove down the side of the lucite plunger which houses the electrode (Yellow Springs Instrument Model 53

Biological Oxygen Moniter). Upon entering the bottom of the sample chamber, the effluent was thoroughly mixed by the continuous operation of a magnetic stirrer and measured for O_2 saturation[2]. The volume of solution within the sample chamber was kept small (less than 2 ml) to minimize the equilibration time at a perfusion rate of flow of about 1 ml min^{-1}. As the effluent was pumped into the chamber for measurement, displaced solution flowed out of sample chamber by way of the access groove of the lucite plunger and was removed from around the top of the apparatus by vacuum suction.

Baseline measurements of O_2 saturation of the fully equilibrated HPB solution were obtained before and after perfusion of the tissue by pumping the HPB in parallel to the tissue perfusion system through the same type, size and length of tubing used in the perfusion system, and at the same rate of flow used for perfusion. The initial baseline value was set to 100% saturation. A continuous record of O_2 saturation levels of the HPB and of the effluent from perfusion were obtained on a Honeywell Electronik 19 chart recorder operated at a 100 mV span.

A flow diagram of the tissue perfusion apparatus is presented in Figure IV.1.

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[2] Care must be taken in order to avoid any introduction of air or other gas into the sample chamber, as this will affect the performance of the electrode.

E. Experimental procedure

Two experiments were carried out in the course of these studies. Both shared common initial conditions. Respiration rate of the mounted tissue was measured when initial chamber solution was placed in the lumen compartments. Then in preparation for inclusion of the anaerobic solution, the chamber was drained and gassed with N_2 for several minutes. When the O_2 saturation of the venous effluent reached an equilibrium, the gas stream was disconnected and the anaerobic buffer was infused into the lumen compartments of the perfusion chamber.

Experiment I

All additions to, or substitutions of, the solutions in the lumen compartments were made after a period of at least 5 min during which the O_2 saturation of the effluent was not changing. In Experiment I, the effects of a high population of rumen microorganisms in the lumen compartments of the chamber were investigated. The anaerobic chamber solution was first replaced by a boiled preparation of resuspended rumen particles and microorganisms and this was subsequently replaced with an unboiled portion of the same suspension.

The rumen particle suspensions were prepared from freshly collected rumen contents from a fistulated steer on a grass hay diet. The contents were strained through four layers of cheesecloth and eight 35 ml portions of the filtrate were centrifuged at 20000 X g for 20 min (2 °C).

The supernatant was discarded and the pellets were randomly allocated into two groups (boiled and unboiled) and each of the groups suspended in 22 ml of anaerobic chamber solution (38 °C). One of the suspensions was heated in boiling water and maintained at a temperature of at least 90 °C for 10 min. It was then allowed to cool to 38 °C, checked for pH, and infused into the emptied lumen compartments of the perfusion chamber. The other suspension was prepared from the sediment immediately before use, checked for pH and used to replace the boiled preparation which had been just previously drained out of the lumen compartments.

The tissue was subjected to two more treatments before termination of the experimental run. Butyrate was added to the unboiled preparation of rumen particles to make a final concentration of 8 mM in the lumen compartments. Mixing of the solution in the lumen compartments was accomplished by its withdrawal and reinjection. This was carried out during the course of the perfusion after additions or changes made to the lumen conditions and in 5 min intervals thereafter, in order to ensure complete distribution of substrates and to minimize sedimentation of contents within the chamber. After addition of butyrate and achievement of effluent saturation equilibrium, propionate was added to the lumen compartments to a final concentration of 22 mM.

Butyrate and propionate were added from stock solutions of HPB plus substrate adjusted to pH 7.1 and maintained at 38 °C. The butyrate and propionate stock solution

concentrations were 160 mM and 440 mM, respectively.

The Experiment I procedure was repeated to yield complete results for 6 pieces of tissue from 6 different animals on separate days.

Experiment II

The second set of experiments differed from the first by inclusion of the substrates before testing the effects of boiled and unboiled strained rumen content preparations. Butyrate, propionate, and then D,L-3-hydroxybutyrate were added to the anaerobic chamber solution to make substrate concentrations in the lumen compartments of 8 mM butyrate, 8mM butyrate plus 22 mM propionate, and 8 mM butyrate plus 22 mM propionate plus 8mM D,L-3-hydroxybutyrate after each successive addition. These were all added from HPB stock solutions (pH 7.1) as described above. When O_2 saturation measurements had reached equilibrium after the organic acid additions, the solution was removed from the lumen and immediately replaced with a boiled suspension of rumen particles containing 8 mM butyrate, 22 mM propionate and 8 mM D,L-3-hydroxybutyrate.

The suspensions were prepared in a slightly different manner compared to suspensions in Experiment I. The rumen contents were obtained from the same animal given the same diet, strained and centrifuged once in a similar manner, but then each pellet was suspended in 15 ml of anaerobic buffer, recentrifuged at the same speed and duration, and the two

groups (boiled and unboiled) were suspended in 22 ml of anaerobic buffer. One preparation was heated in boiling water and maintained at a temperature of 90 °C for at least 10 minutes. The washed sediment preparation was then allowed to cool to 40 °C and butyrate, propionate, and 3-hydroxybutyrate were added to make a final concentration of 8, 22, and 8 mM respectively. The pH at 38 °C was measured before placing the boiled preparation plus substrates in the lumen compartments. The unboiled preparation was subjected to the same procedure with the exception of the heating and was substituted for the boiled preparation after effluent O_2 content was stabilized.

Results were obtained for 7 pieces of tissue from 7 animals on separate days.

Termination of perfusion

Before perfusion was terminated, rate of venous effluent flow was measured over a 5 min interval. Any perfusions in which the effluent collected was less than 90% of the pump rate were discarded. The arterial catheter was then disconnected from the pumphead and the venous catheter taken out of the O_2 electrode chamber. The HPB solution was directed to the O_2 electrode chamber for baseline measurements of the HPB O_2 saturation. Most experimental runs were 2 to 3 h maximum duration and it was rare for the O_2 saturation of the HPB to drift more than a few percentage units in saturation between the start and the finish of the

run.

As a check on the effectiveness of the perfusion, 1 ml of 150 mM Evan's Blue dye (pH 7.1) was injected into the clamped tissue at a rate of 1 ml min^{-1} via the arterial catheter. The flow of the dye also allowed a visual check for obvious and quantitatively important points of perfusate leakage. After administration of dye, the chamber was opened to observe the tissue. The results from any tissue that had not stained completely throughout the perfusion area were not considered satisfactory and were discarded. The stained area was then cut away from the remainder of the tissue and dried at 80 °C to a constant weight. The area of any tissue taken for post-perfusion tissue samples (see below) was carefully measured and subsequently related back to dry weight of tissue perfused.

Electron microscopy samples

Samples (4 mm X 6 mm) from 3 of the omasal leaves perfused in Experiment II were taken for observation of bacteria adhering to the mucosal wall. Samples were collected and prepared for investigation by both scanning and transmission electron microscopy (SEM and TEM)[3]. Three samples of tissue were fixed per leaf, each being taken at various stages of collection and perfusion. Two samples were

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[3] SEM observations on omasal leaf samples were performed by Mr. G. Braybrook, Department of Entomology, University of Alberta, and TEM observations were made by Dr. J.W. Costerton, Department of Biology, University of Calgary.

taken adjacent to the area of perfusion; one of them was fixed immediately upon collection at the abattoir (fixed at plant) and the other was taken and fixed just prior to mounting the tissue within the perfusion chamber (preperfusion). The postperfusion sample was obtained from the perfused area after termination of the perfusion. Preperfusion and postperfusion samples were split into two halves, one half being fixed with 5% glutaraldehyde in 0.067 M cacodylate buffer (pH 7.2) for SEM investigation and the other half being fixed in the same fixative with added ruthenium red (pH 7.2) for TEM observation. The samples collected and fixed at the abattoir were prepared for SEM observation only. All of the samples remained in the fixative for 2 h. Following the 2 h period in fixative, all samples were washed twice in 0.067 M cacodylate buffer (pH 7.2; with or without ruthenium red, depending on which fixative was used upon the samples) for 15 min per wash and stored at 4 °C in the buffer. Samples to be observed by SEM were dehydrated in a graded series of ethanol washings, critical point dried, mounted on metal observation stubs, and gold coated 24 h before observation. SEM samples were scanned on a Cambridge Stereoscan 180 at an accelerating voltage of 20 kV to assess the presence of bacteria adhering to the epithelium. TEM samples were shipped to the University of Calgary in 0.067 cacodylate buffer plus ruthenium red, and dried and embedded for evaluation of bacterial attachment to the epithelium.

F. Calculations

Respiration rates of the tissue were calculated as nmoles of O_2 consumed per mg of tissue dry weight per h. The saturated level of O_2 in the HPB was considered to be 800 nmoles ml^{-1}. This value was selected for several reasons:

1. the solubility of O_2 in Ringers solution at one atm of pressure of O_2 was reported as 0.0245 ml O_2 ml^{-1} at 35 °C and 0.023 ml O_2 ml^{-1} at 40 °C (Umbriet et al. 1964). Interpolation of these values indicates solubility of 0.0236 ml O_2 ml^{-1}.
2. use of the Ideal Gas Law to convert volume of O_2 in 1. (above) to moles of O_2 per ml of Ringers solution results in a value of 925 nmoles O_2 ml^{-1} of solution.
3. since the HPB is equilibrated with 95% O_2: 5% CO_2 at 38 °C, and the prevailing barometric pressure in Edmonton is about 700 mmHg, the pO_2 in and above solution would be expected to to be about 665 mmHg. In comparison with Ringers solution at a pO_2 of 760 mmHg, one might expect that the solubility of O_2 in the HPB should be 665/760 X 925 = 809 nmoles O_2 ml^{-1}.

Since the O_2 saturation of the effluent is the parameter measured and recorded, the O_2 extracted by the tissue is the difference between the saturation level of the effluent and the saturation level of the fully oxygenated buffer (800 nmoles O_2 ml^{-1}). The addition of substrate would be expected to increase the respiration rate of the tissue

and therefore result in a decrease of O_2 saturation in the venous effluent. In order to remain consistent, the measure of difference between the oxygenated buffer and the effluent was taken at the lowest saturation level recorded for a period of at least 5 min. If the drop (or increase) in saturation of the effluent was only transient, this was not used in calculation of respiration rate. In addition, any drift in the saturation level recorded for the oxygenated HPB from the beginning to the end of the experimental run, was assumed to have been linear over time and differences between the effluent and the HPB were calculated on this basis. For example, if the experimental run lasted 2 h, and the saturation level of the HPB solution was set at 100% at the start of the run and was measured at 96% at the end of the run, then half of the way through the run, the saturation level was assumed at 98%. If the effluent saturation at this time was 48%, the rate of flow through the tissue was 0.96 ml min^{-1}, and the tissue dry weight was 200 mg, O_2 consumption of the tissue was calculated as:

$$=(0.98-0.48) \times 800 \text{ nmoles ml}^{-1} \times 0.96 \text{ ml min}^{-1} \times 60 \text{ min h}^{-1} \times 200 \text{ mg}^{-1}$$

$$=115 \text{ nmoles } O_2 \text{ (mg dry weight)}^{-1} \text{ h}^{-1}$$

It was decided to calculate and present O_2 consumption as nmoles rather than as volume (microliters) however conversion between nmoles used and volume can be obtained

with the use of the Ideal Gas Law.

G. Statistical analyses

For both experiments I and II, the data was analyzed by a two-way analysis of variance. The main effects were attributed to variation caused by animals (one tissue studied per animal) and to variation caused by treatment. Since there was only one observation per treatment per animal (tissue), the error term was described by the animal by treatment interaction. This experimental design is described as a complete block design (Steel and Torrie 1980). Means of treatment effects were tested using the Duncan-Multiple-Range test at the 0.05 level of significance when a significant F-value indicated treatment differences (Steel and Torrie 1980).

Table IV.1 Perfusion and lumen chamber media

	Final concentration mM
Sodium chloride	108.0
Potassium chloride	4.7
Calcium chloride	3.0
Magnesium sulfate	1.2
Potassium phosphate, monobasic	1.2
Ethylenediamine tetraacetic acid disodium salt	0.5
Hepes[1]	25.0
Sodium bicarbonate	10.0
Nitrogycerin[2]	1.2 ng/ml
Resazurin[3]	0.11 mg/l

[1] N-2-hydroxyethylpiperazine-N-2-ethane-sulfonic acid
[2] used in perfusion buffer only.
[3] used in the lumen solutions only.

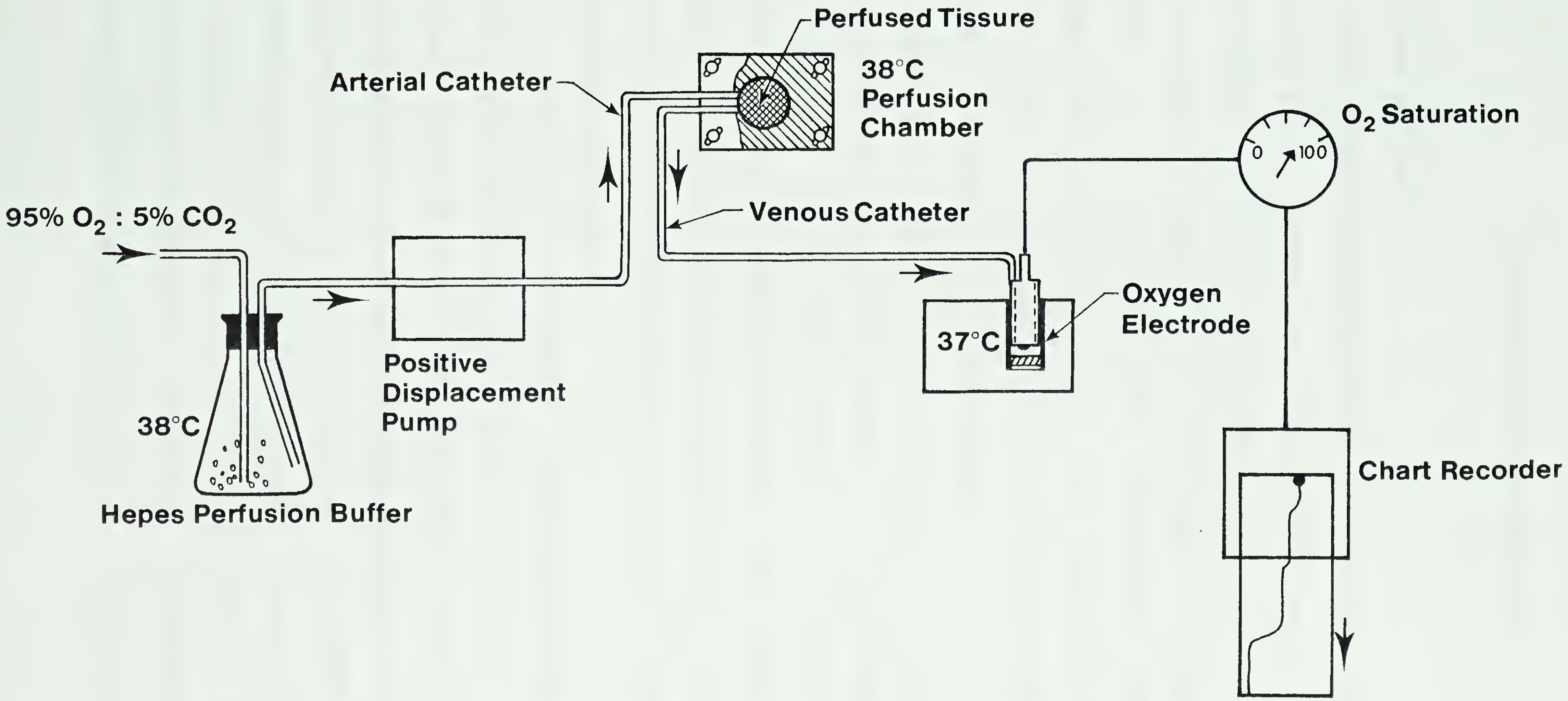
Perfused Tissure
Arterial Catheter
38°C
Perfusion
Chamber
O_2 Saturation
0
100
95% O_2 : 5% CO_2
Venous Catheter
Oxygen
Electrode
37°C
Positive
Displacement
Pump
38°C
Hepes Perfusion Buffer
Chart Recorder

Figure IV.1 Flow diagram of tissue perfusion and oxygen saturation measurement.

V. RESULTS AND DISCUSSION

A. Tissue perfusion

Observations and measurements obtained from vascular perfusion of the omasal leaf indicated that this was a valid preparation in which to study the respiration and metabolism of the tissue. In properly catheterized and mounted tissue, the flow rate of venous effluent remained at 0.95 - 1.00 of the pump infusion rate. Little tissue edema was evident and the only location of perfusate leakage was at the point of catheter insertion into the vessels.

Further indication of the validity of the preparation was provided by the responsiveness of O_2 utilization to conditions within the lumen chambers. O_2 removal reacted quickly to changing conditions within the lumen chambers and remained in equilibrium while conditions were not changing. O_2 utilization did not respond to addition of substrates above saturating levels.

In addition, O_2 withdrawal from the perfusate could be partially abolished by inclusion of 10^{-3} - 10^{-2} M NaCN in the lumen chambers and in the perfusate. On the two occasions tested, NaCN was introduced at the end of the perfusion. In both instances, cyanide resulted in an O_2 utilization rate of 0.35 of the rate initially experienced by the tissue (initial buffer; no substrates). In neither case did cyanide completely abolish O_2 withdrawal from the perfusate while it passed through the tissue and in both

cases it took almost 2 h for the cyanide to gradually manifest its full effect. One may conclude that either 10^{-3} - 10^{-2} M NaCN was only partially effective in poisoning the tissue or perhaps a certain proportion of the O_2 removed from the perfusate was not used for metabolic purposes, but was simply being lost by diffusion out of solution into the surrounding tissue and chamber media. It was noted that the effect of cyanide was gradual, indicating that the epithelium may be partially impermeable or insensitive to the action of this potent cytochrome oxidase inhibitor.

Another factor which may have contributed to the partial ineffectiveness of cyanide may be related to the effect of cyanide on the bacteria within the rumen particle suspension. Since cyanide administration at the end of the trial was made into the lumen chambers which contained an unboiled and unwashed rumen particle suspension, perhaps the cyanide was not effective in inhibiting the utilization of O_2 by the microorganisms present in the suspension. According to Jurtshuk and Yang (1980), it is rare for a bacterial species to have a single cytochrome oxidase serve as the only, terminal, O_2-reactive component in electron transport. Thus differing electron transport systems may have differing sensitivities to cyanide. In addition, cyanide will not inhibit the electron transfer from NADH to O_2 to form peroxide catalyzed by NADH oxidase (Knowles 1980; McCord et al. 1971). NADH oxidase activity in rumen bacteria is considerable (Hobson and Wallace 1982b) and rumen

bacteria seem capable of utilizing considerable amounts of O_2 without a significant effect on redox potential (Baldwin and Emery 1960). Baldwin and Emery (1960), Czerkawski (1972), Cheng et al. (1981), and Hobson and Wallace (1982b) suggested that O_2 utilization in the rumen is more commonplace than traditionally accepted, and that the utilization of O_2 by facultative organisms may preserve the correct environment for the anaerobic microbes. Thus it seems possible that the O_2 withdrawn from the perfusate during exposure to NaCN may be at least partially attributable to uptake by the bacteria in the lumen chambers.

The effectiveness of perfusion was indicated by the complete staining of the isolated tissue area by Evans Blue dye. The dye did not appear in areas outside of that clamped within the chamber and it was distributed evenly within the isolated circular area. The even distribution of dye must indicate a complex system of anastomoses within omasal leaves. Branches of the radial vessels of the omasal leaves freely anastomose with each other and therefore provide several points of supply to the subepithelial capillary bed (Comline et al. 1968), which no doubt aided in the successful perfusion of the circular area clamped in the chamber.

B. O_2 extraction from perfusate during initial solution, anaerobic solution, and N_2 exposure

Data from Experiment I is presented in Table V.1 and a theoretical example of the recorded O_2 saturation levels during the conditions imposed in Experiment I is presented in Figure V.1. Figure V.1 is constructed in order to represent the mean treatment values (with the standard error of the treatment mean) obtained in Experiment I for a tissue experiencing a rate of flow of 1 ml min^{-1} and having a dry weight of 250 mg; i.e. O_2 consumption rate during initial solution exposure was

$$(1.00-0.72) \times 1.0 \text{ ml min}^{-1} \times 800 \text{ nmoles } O_2 \text{ ml}^{-1} \times 60 \text{ min h}^{-1} \times 250 \text{ mg}^{-1}$$
$$=54 \text{ nmoles } O_2 \text{ (mg dry weight)}^{-1} \text{ h}^{-1}$$

which corresponds to the treatment mean obtained for the initial solution in Experiment I (Table V.1). Results of Experiment II are presented in an identical manner in Table V.2 and Figure V.2.

The first three conditions imposed upon the lumen side of the tissue are common to both experiments. It can be observed from Tables V.1 and V.2 and Figures V.1 and V.2 that these treatments resulted in similar trends of O_2 withdrawal from the perfusate as it passed through the tissue. In both experiments, the initial and anaerobic chamber solutions resulted in significantly lower mean O_2

consumption rates ($p \leq 0.05$) than the rates induced by the presence of N_2 in the lumen chambers. The mean respiration rates obtained with the initial and anaerobic solutions did not differ significantly ($p > 0.05$) in either experiment, however the extraction of O_2 from the perfusate in the presence of anaerobic solution was consistently greater than O_2 extraction with initial solution in the lumen chamber.

In earlier reference to tissue treatment with NaCN, it was indicated that a major proportion of the initial O_2 consumption rate appeared due to metabolic activity. Previous in vitro studies have illustrated the presence of substantial endogenous metabolism by forestomach epithelium. Pennington (1954) reported endogenous (no added substrate) O_2 consumption values of 77 - 134 nmoles O_2 (mg dry weight)$^{-1}$ h^{-1} in ovine rumen epithelium collected from a slaughterhouse and held in ice-cold Krebs-Ringer buffer for 2 h before incubation. Hird and Symons (1959) reported similar values for pieces of bovine omasal laminae which were handled similarily with the interval between collecton and start of incubation being 40 - 80 min. Giesecke et al. (1979) measured similar endogenous respiration rates in incubated rumen epithelium from 6-month-old lambs. The values observed with the initial or anaerobic solutions are also in the range of endogenous respiration rates previously reported for omasal epithelium (Hird and Symons 1959) and slightly lower than most of the values obtained with stripped rumen epithelium (rumen wall with the musculature

removed) from sheep or cattle (Pennington 1954; Goosen 1976; Giesecke et al. 1979).

In Experiments I and II, the endogenous respiration (O_2 consumption rate in the presence of initial or anaerobic solution with no added substrates) accounted for over half of the maximum O_2 consumption observed for perfused omasal leaves. This is a consistent observation across numerous studies of respiration of rumen or omasal epithelium, regardless of the substrate or substrates added to the incubation medium (Pennington 1954; Hird and Symons 1959; Goosen 1976; Giesecke et al. 1979). This high endogenous respiration persists even after relatively long periods between collection and incubation with substrate. Analysis of extracts of rumen epithelium collected from fasted adult cows revealed a mean content of 0.24 μmoles volatile acid cm^{-2} of rumen wall area which shows the presence of substantial endogenous substrate (Stevens and Stettler 1966a). In addition, rumen papillae incubated in the absence of substrate continue to secrete significant quantities of lactate, acetoacetate and 3-hydroxybutyrate (Weigand et al. 1975; Giesecke et al. 1979). Goosen (1976) attributed the high endogenous respiration to carbohydrate or glycogen metabolism in the rumen epithelium, however ketone body secretion in the absence of substrate indicates metabolism of ketogenic substrates in the epithelium. Also, Emmanuel (1981b) reported only low levels of glycogen in the rumen epithelium from adult sheep and cattle. The glycogen content

of forestomach epithelium from young ruminants was several fold greater than for mature animals (Emmanuel 1981b), which is an observation consistent with the propensity to metabolize glucose exhibited by the developing forestomach epithelium (Fell and Weekes 1975; Giesecke et al. 1979). In regard to the perfused omasal leaf, the age of the animal could have an effect on the endogenous rate of O_2 utilization.

It is interesting to note that while not significantly different from the initial solution ($p>0.05$), the O_2 utilization rate with an anaerobic solution on the lumen side was consistently greater. During initial perfusion, it is probable that O_2 was being extracted from the chamber solution as well as the perfusate, since boiling did not eliminate all of the O_2 from the chamber solution. O_2 withdrawal from the lumen solution would not be included in the O_2 consumption calculated from perfusion effluent saturation. Consequently, O_2 utilization in the presence of anaerobic solution is probably a better measure of endogenous respiration and subsequent reference to endogenous O_2 utilization will pertain to O_2 consumption during exposure to anaerobic solution with no substrates.

The dramatic increase in O_2 extraction from the perfusate during N_2 gassing is a difficult phenomenon to explain. N_2 insufflation into the reticulorumen of a cow had no effect on rumen arterial blood flow (Sellers 1965). Replacement of N_2 with CO_2 to elevate pCO_2 in the

reticulorumen of sheep resulted in increased subepithelial blood flow in the reticulum and rumen wall (Dobson 1979). This would seem to indicate that CO_2 has a larger physiological effect on blood flow than N_2.

In virtually every tissue successfully perfused, administration of the anaerobic buffer, without substrates, into the lumen chambers resulted in a substantial decrease in O_2 extraction from the perfusate as compared to the O_2 extracted during N_2 gassing. The mean reduction in O_2 consumption was 35 and 42% for Experiments I and II respectively. If there was simply diffusion of O_2 across the tissue to the lumen side, perhaps the N_2 atmosphere in the chamber provided a more effective gradient for O_2 diffusion than the N_2-equilibriated anaerobic buffer. The diffusion coefficient of O_2 in N_2 is 0.181 $cm^2\ sec^{-1}$ (Am. Inst. Physics Handbook 1972) while the coefficient in water is 10^4 fold smaller (1.8×10^{-5} $cm^2\ sec^{-1}$ in 20°C water; Ramm 1968), therefore it would be expected that after crossing the epithelium, O_2 would move away and maintain a greater gradient with N_2 than with anaerobic buffer in the chamber. The greater diffusion gradient for O_2 out of the epithelium may have triggered perfusion of a greater number of subepithelial capillaries. It has been well documented that interstitial pO_2 is a microvascular regulator within the small intestine. A drop in local pO_2 in small intestinal tissue may cause a dramatic shift in blood flow not only by vasodilation of precapillary sphincters and perfusion of

more capillaries in the mucosa, but also by affecting distribution arterioles in the mucosa and submucosa causing a general increase in blood flow through the mucosal microvasculature (Bohlen 1980; Granger et al. 1980; Shepherd 1982). One could speculate that a similar physiological control exists in the forestomach wall, in which case pO_2 in the tissue was depressed as a result of the diffusion gradient promoted by N_2.

If the movement of O_2 out of forestomach epithelium persists in vivo, obvious implications arise in respect to fermentation in the rumen. Cheng and Costerton (1980) have already inferred that O_2 diffusion across the rumen wall may be quantitatively important since 0.25 to 0.50 of the adherent rumen wall microbial population is composed of facultative anaerobes. They have suggested that these microbes have an "oxygen scavenging" role in the rumen, and could be in a position to transfer reducing power generated in the rumen to O_2. This will be discussed in greater detail later in the chapter.

C. Experiment I

Replacement of the anaerobic chamber solution with the boiled preparation of rumen particles resulted in a 35% increase ($p \leq 0.05$) in the O_2 utilization rate. The primary objective of this inclusion into the lumen chamber was to obtain a concentrated but metabolically inactivated population of rumen bacteria while minimizing additional

changes in the lumen chamber conditions.

The more rigid and less degradable plant tissues require direct adherence by specific types of rumen bacteria for digestion to proceed (Akin 1979), and therefore a large proportion of rumen bacteria are associated with particles in the rumen (Cheng and Costerton 1980; Hobson and Wallace 1982a). Although some of the bacteria associated with plant material are lost when rumen contents are strained through cheesecloth (Cheng et al. 1979), free living bacteria and bacteria associated with the smaller rumen particles (≤1 mm) are retained in the preparation. Despite being heated, the rumen particle preparation prompted a 35% increase in O_2 extraction ($p \leq 0.05$) from the perfusate. Some of the increase may be attributed to the presence of substrate in the preparation, since the rumen particles were not washed before being suspended for addition into the lumen chamber. This possibility is strengthened by the lack of stimulation of tissue O_2 consumption upon addition of butyrate or propionate to the unboiled rumen particle preparation. It is possible that a proportion of the increase brought about by the boiled preparation was caused by a factor or factors associated with the rumen particles or the rumen microbes. However, the presence of bacteria and other components of the rumen fluid sediment which were not exposed to heat did not consistently increase O_2 utilization of the tissue over that observed with the boiled rumen particle preparation ($p > 0.05$) (Table V.1). Thus most of the increase in O_2

utilization conferred by the rumen particle preparations was due a heat-stable component, or components, of the suspensions.

The mean pH values of the anaerobic solution, the boiled rumen particle preparations and the unboiled rumen particle preparations were 7.16±0.014, 6.92±0.114 and 6.67±0.079 respectively ($p \leq 0.05$). In preliminary perfusions performed with boiled lumen buffer plus butyrate (8 mM) and propionate (22 mM), adjustment of the lumen pH by up to 1 pH unit with dilute HCl did not induce changes in O_2 utilization rate. Therefore in spite of the differing mean pH values, it is unlikely that pH was involved in any stimulation of O_2 consumption.

It appeared as if the unwashed rumen particle preparations contained sufficient volatile fatty acids at levels such that the tissue was saturated with substrate and respiration was unresponsive to addition of butyrate (8 mM) and then propionate (22 mM) (Table V.1 and Figure V.1). Literature reports (Pennington 1954; Goosens 1976; Giesecke et al. 1979) of marked respiration responses to butyrate in incubated rumen epithelium suggest that the levels of butyrate in the rumen particle suspensions were sufficient to make a further addition uneffective. The addition of 22 mM propionate after butyrate did not elicit any further increase in O_2 utilization. However, propionate has not caused appreciable increases in O_2 uptake in incubated rumen epithelium in previous reports in the literature (Pennington

1954; Goosen 1976; Giesecke et al. 1979).

D. Experiment II

The results of Experiment II are presented in Table V.2 and Figure V.2 in the same manner as results of Experiment I. Endogenous respiration with boiled lumen buffer, N_2 gas and anaerobic lumen buffer were carried out in the same manner as in Experiment I and are discussed in section B of this chapter.

Experiment II was designed to supplement observations made in Experiment I, therefore substrates were added to the anaerobic chamber solution followed by a washed preparation of rumen particles containing substrates at the same concentration as in the anaerobic solution. The rumen particles had been washed in anaerobic solution in order to ensure the removal of the majority of soluble substrates and substances from the rumen particle preparations.

Inclusion of 8 mM butyrate resulted in a mean increase of 31% ($p \leq 0.05$) as compared to endogenous O_2 utilization with anaerobic buffer on the lumen side. Subsequent additions of propionate (22 mM) and the ketone body, D,L-3-hydroxybutyrate (8 mM), had no significant effect ($p > 0.05$) on O_2 removal. The inability of D,L-3-hydroxybutyrate to promote a greater rate of respiration suggests a limited conversion of D-3-hydroxybutyrate to acetoacetate in omasal epithelium in the presence of butyrate and propionate. This conversion

would be catalyzed by D(-)-3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) which would generate NADH and acetoacetate as products of the reaction. The enzyme is present in forestomach epithelium (Koundakjian and Snoswell 1970; Watson and Lindsay 1972; Chandrasena et al. 1979) but results of Experiment II would indicate either negligible activity in the direction of acetoacetate formation, or if there was activity, it was offset by decreased oxidation of another substrate or did not entail transfer of electrons to O_2.

Replacement of the anaerobic solution plus substrates with the boiled preparation of washed rumen particles containing substrates resulted in a 18% increase ($p \leq 0.05$) in O_2 utilization (Table V.2 and Figure V.2). The significant increase in O_2 consumption rate upon inclusion of the boiled rumen particle preparation plus substrates indicated that there are heat-stable factors within the preparation which are either increasing the metabolic activity of the tissue (or the metabolic activity of bacteria adhering to the tissue) or promoting a greater loss of O_2 from the perfusate. The unboiled washed rumen particle preparation caused a further 12% increase ($p \leq 0.05$) in O_2 utilization to the point where the O_2 used in this condition was significantly greater than for any other treatments imposed (Table V.2). Thus there was an additional interaction of O_2 removal from the perfusate with a heat-labile component of the washed rumen particle suspension.

The mean pH values of the anaerobic solution, the boiled rumen particle suspensions and the unboiled rumen particle suspensions within Experiment II were 7.18±0.015, 6.85±0.095 and 6.72±0.097 respectively[4]. The mean pH of the rumen particle suspensions differed significantly from the anaerobic solution ($p \leq 0.05$), but were not significantly different from each other ($p > 0.05$). As referred to in Section C, it is unlikely that the pH of the lumen chamber contents had a significant effect on tissue O_2 consumption.

E. Electron microscopy samples

SEM and TEM observation revealed the presence of adherent bacteria on the tissue both before and after perfusion (Figures V.3 - V.6). Bacteria were primarily located in microprotected areas of the epithelial surface, with the lowest densities observed on the tips of the papillae. Scoring of the bacterial populations adhering to the epithelium according to the method of McCowan et al. (1980) revealed scores of 0 - 1 on the crests of the omasal papillae and 1 - 3 in areas between the papillae in both preperfusion and postperfusion samples. These scores correspond to very low populations of adhering bacteria on the papillae tips while coverage in the more unexposed areas of the epithelium varied from about 10 - 50% of the surface

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[4] The mean pH of the lumen chamber media and rumen particle preparations were calculated from five of the seven perfusion trials due to missing measurements in two of the trials.

area. TEM revealed that most of the bacteria adhering to the epithelium in preperfusion and postperfusion samples were tightly attached to the tissue. SEM observations on the abattoir-fixed samples usually revealed substantially more coverage of bacteria on the tissue than were seen on the perfusion samples (Figures V.7 and V.8). Therefore it appeared as if some of the loosely adhering bacteria had been washed off during transport and preparation for perfusion. There were no indications of net attachment or disattachment during the perfusion.

F. General discussion

The results of both Experiments I and II indicate that lumen conditions may drastically affect the withdrawal of O_2 from the perfusate as it passes through the omasal leaf. Not only is the O_2 utilization affected by substrate provided from the lumen side of the omasal tissue, but it is also affected by N_2 and by a concentrated suspension of rumen particles and microorganisms. Although the conditions imposed within the lumen chamber were not identical to those in vivo, the fact that the lumen was kept anaerobic and O_2 was provided by perfusion permitted possible insights into metabolic interactions between the animal and the forestomach wall that have not previously been achieved.

Enhancement of O_2 removal by butyrate was consistent with the published evidence of butyrate metabolism in the forestomach epithelium. Likely 0.85 - 0.9 of the butyrate

absorbed through the forestomach wall will be metabolized (Stevens 1970; Bergman 1975). Evidence also exists for propionate metabolism in this tissue (Stevens 1970; Bergman 1975; Weekes and Webster 1975). However the results obtained with perfused omasal leaves agree with previous reports that propionate does not appreciably elevate respiration in the presence of butyrate above the respiration rate induced by butyrate (Goosen 1976).

Comparison of O_2 removal in the presence of boiled and unboiled rumen particle suspensions to the endogenous O_2 utilization rate with anaerobic solution in the lumen reveals that the boiled preparations in Experiments I and II resulted in increases of 35 and 55% respectively. The rumen particle preparations in Experiment I did not include added substrates, however because the suspensions in Experiment I were unwashed and did not respond to added substrates, it is presumed that the boiled and unboiled suspensions contained saturating levels of substrate. The unboiled preparations in Experiments I and II resulted in increases of 50 and 73% respectively, above the endogenous O_2 utilization rates. Of the total increase in O_2 consumption stimulated by the rumen particle suspensions (plus substrates), 70 - 75% of the increase was due to the heat-stable component of the rumen particle suspension plus substrates, and the remaining 25 - 30% was due to the heat-labile component. The data from Experiment I does not allow an estimate of the contribution of the butyrate in the particle suspension to the total

increase above the endogenous rate, however from the results of Experiment II, 42% of the total increase in O_2 utilization caused by unboiled, washed rumen particle and microorganisms plus substrates is due to addition of butyrate, 32% is due to the heat-stable component(s) and 25% is due to the heat-labile component(s) of the rumen particle suspension.

The rumen particle preparations contain a multitude of constituents and it is, therefore, difficult to single out a particular component or components within the suspension which provoke a greater O_2 utilization. However the most obvious heat-labile constituent within the suspensions is the microorganisms, which might directly cause an increase of metabolic activity of the omasal epithelium by some unknown means, or may effect a greater O_2 removal due to their own respiration. One would expect that microbial O_2 uptake, if it occurs, would be largely limited to those microorganisms located in close proximity to the epithelium. Cheng and Costerton (1980) and Cheng et al. (1981) have, in fact, proposed several functions for bacteria adherent to the ruminant forestomach epithelium, one of which is O_2 scavenging. Czerkawski and Breckenridge (1969) have reported that rumen fluid microorganisms incubated with sucrose did not exhibit any significant effects on CO_2, VFA and organic matter production even with reasonably high continuous infusion rates of O_2 into their artificial rumen. These authors concluded that O_2 introduction into the rumen would

not produce deleterious effects on rumen fermentation and may prove to be beneficial to energy metabolism in the rumen.

The bacteria adhering to the epithelium upon commencement of the perfusion could certainly have contributed to the endogenous O_2 utilization since they may be obtaining some of their substrates from the dead cell layer of the epithelium (Wallace et al. 1979; Cheng and Costerton 1980). Wallace et al. (1979) reported that a normal population of bacteria adherent to rumen epithelium was maintained in growing lambs during a 6-month period in which the only nutrients administered to the animals were a mixture of VFA in bicarbonate buffer infused into the rumen and casein (and other esential nutrients) infused into the abomasum. This indicated that the microbes adhering to the rumen wall were obtaining supplementary nutrients from the epithelium or across the epithelium. In addition, 25 - 50% of the microbes identified on the reticulorumen wall are facultative anaerobes (Cheng and Costerton 1980) which is consistent with the suggestion that the adherent bacteria may be contributing to endogenous O_2 utilization.

No increase or decrease in microbial attachment to the omasal epithelium was evident during the course of the perfusion. This was based on subjective scores (McCowan et al. 1980) taken of the bacteria associated with the epithelium both preperfusion and postperfusion. The limited duration of perfusion may have precluded detection of

microbial attachment. However, microorganisms in close proximity may be able to interact with the tissue without needing structural attachment which could explain the increment in O_2 utilization (Experiment II) caused by the heat-labile component(s) of the rumen particle preparation. McCowan et al. (1980) noted that the areas of the rumen experiencing the highest subepithelial blood flow (ventral rumen; Engelhardt and Hales 1977) also exhibited a larger population of ureolytic bacteria. They suggested that the greater blood flow was the reason for greater ureolytic bacterial association with the ventral rumen wall than any other area of the reticulorumen. Areas with relatively high blood flow might be expected to favour establishment of more facultative anaerobes which could utilize O_2 delivered through the epithelium to oxidize many products of dismutation available from fermentation in the rumen.

The nature of the heat-stable component(s) within the rumen particle suspension which stimulate an increase in O_2 utilization are more difficult to identify. The boiled rumen particle suspension may contain structural or chemical properties which stimulate O_2 withdrawal from the perfusate by some unknown mechanism. Perhaps addition of the boiled rumen particle suspensions stimulated the metabolism of the bacteria already present on the epithelium by providing them with feedstuffs and thereby triggering a greater withdrawal of O_2 from the perfusate.

In conclusion, omasal epithelium perfusion has indicated that the withdrawal of O_2 from the perfusate is responsive to conditions on the lumen side of the tissue. This utilization of O_2 is not necessarily due to an increase in tissue metabolism. It may be sensitive to O_2 demand within the lumen of the forestomach. There are components within the rumen contents which either stimulate the respiration rate of the epithelial cells and directly or indirectly stimulate the delivery of O_2 through the epithelium into the lumen. The microbial population adherent to the forestomach wall and in close proximity to the forestomach wall are likely involved in O_2 delivery and utilization at the forestomach epithelium. This may be a very important consideration in regard to overall rumen fermentation efficiency, since these microbes form the interface between the virtually anaerobic rumen contents and the very oxidatively active epithelium.

Table V.1. Oxygen utilization rates: Experiment I[1].

	Perfusion no.							
	1	2	3	4	5	6	Mean[2]	S.E.
Lumen chamber conditions								
Initial solution	63.5	57.4	33.1	48.3	67.1	54.5	54.0a	4.98
Nitrogen gas	134.6	85.5	49.1	64.9	134.3	55.9	87.4b	15.70
Anaerobic solution	94.0	65.0	39.1	62.1	77.5	51.8	64.9a	7.85
Boiled rumen particles	114.3	106.0	61.1	84.2	82.6	79.1	87.9b	7.88
Unboiled rumen particles	134.6	118.0	61.1	89.7	103.3	79.1	97.6b	10.89
Plus butyrate (8mM)	132.1	123.1	61.1	89.7	100.7	79.1	97.6b	10.93
Plus propionate (22mM)	134.6	124.8	61.1	89.7	100.7	79.1	98.3b	11.33

[1] All values reported as nmoles oxygen (mg dry weight)$^{-1}$ h^{-1}.

[2] Means with differing letters are significantly different ($p \leq 0.05$).

Table V.2. Oxygen utilization rates: Experiment II[1].

Lumen chamber conditions	Perfusion no. 1	2	3	4	5	6	7	Mean[2]	S.E.
Initial solution	49.6	64.7	50.1	61.2	83.6	82.6	67.3	65.6a	5.19
Nitrogen gas	103.7	88.7	73.7	87.0	112.2	146.8	118.3	104.3bc	9.19
Anaerobic solution	58.6	66.5	57.5	69.3	81.2	107.0	74.2	73.5a	6.41
Plus butyrate (8mM)	76.6	92.4	67.8	88.6	109.8	131.5	106.7	96.2b	8.32
Plus propionate (22mM)	74.4	94.2	70.8	96.7	117.0	131.5	109.0	99.1b	6.32
Plus D,L-3-hydroxybutyrate (8mM)	81.3	86.8	72.3	98.3	117.0	110.1	109.0	96.4b	6.33
Boiled rumen particles[3]	110.4	92.4	76.7	114.4	148.0	116.2	136.8	113.6c	9.19
Unboiled rumen particles[3]	112.7	101.6	88.5	119.2	148.0	168.2	153.1	127.3d	11.14

[1] All values reported as nmoles oxygen (mg dry weight)$^{-1}$ h^{-1}.

[2] Means with differing letters are significantly different ($p \leq 0.05$).

[3] Rumen particle suspensions contained 8 mM butyrate, 22 mM propionate, and 8 mM D,L-3-hydroxybutyrate.

Figure V.1. Oxygen saturation recording: experiment I.

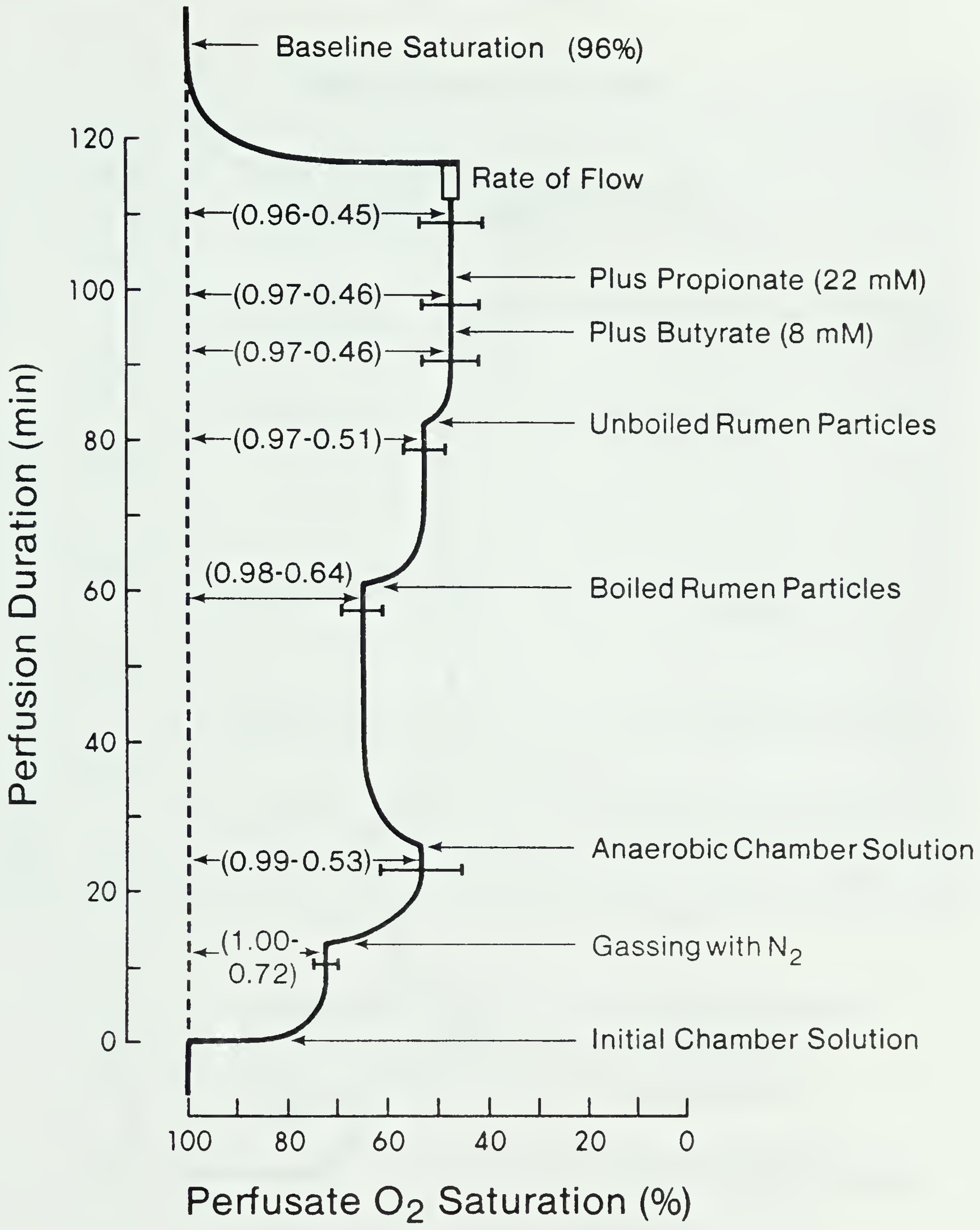
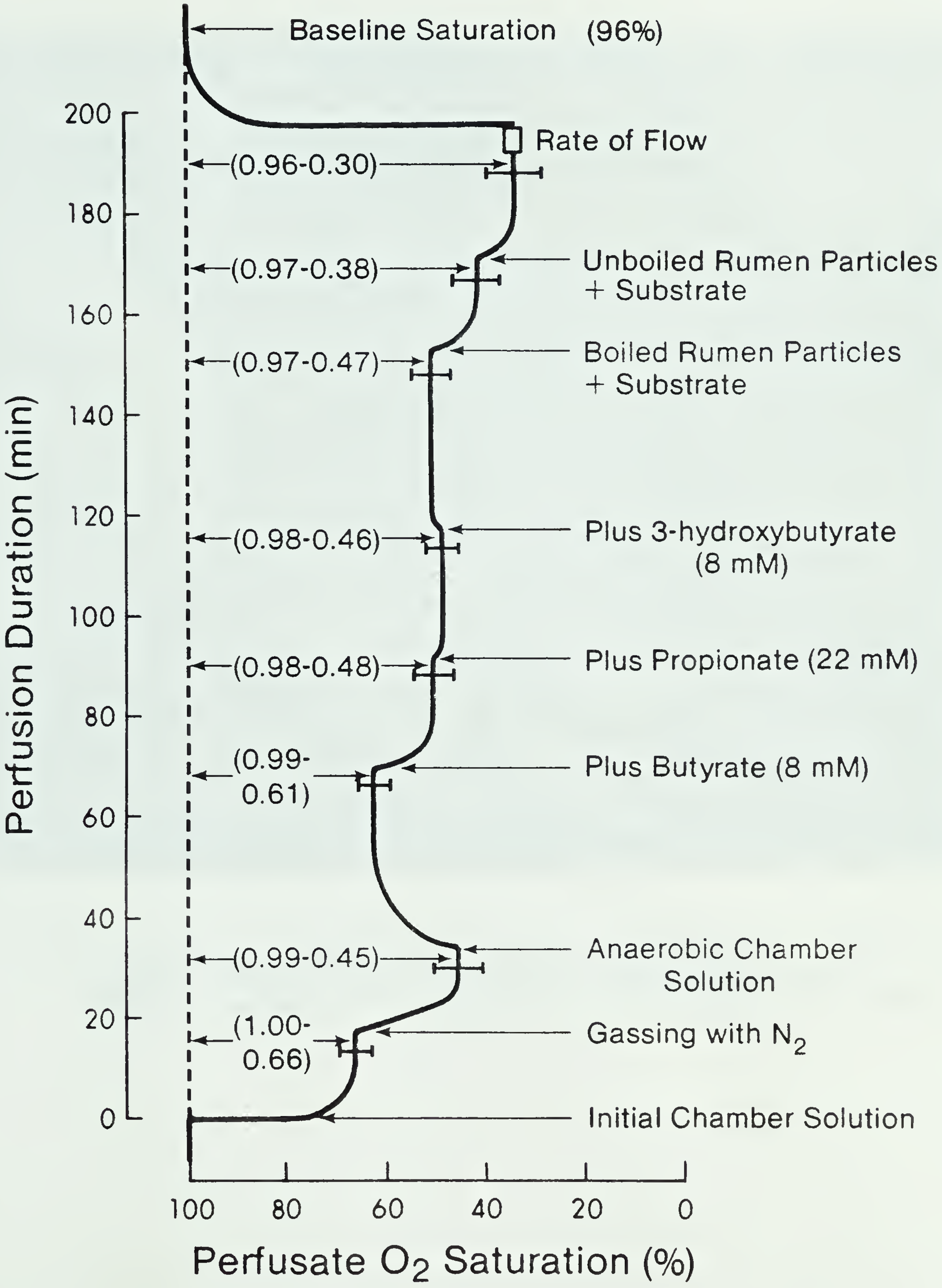


Figure V.2. Oxygen saturation recording: experiment II.



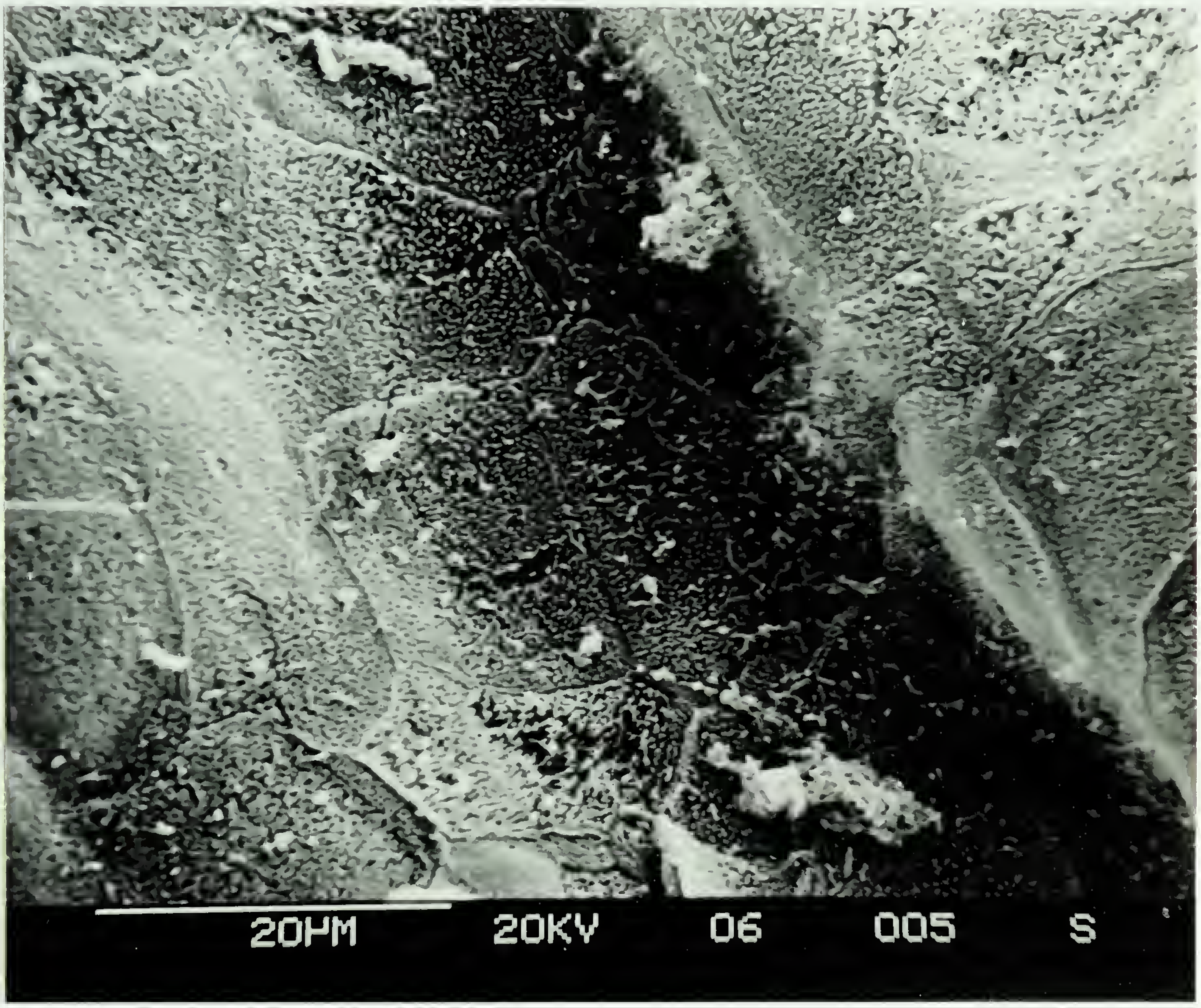


Figure V.3 SEM of omasal epithelium, preperfusion. Note the sparse population of bacteria adhering to the epithelium and the outline of the epithelial cells.

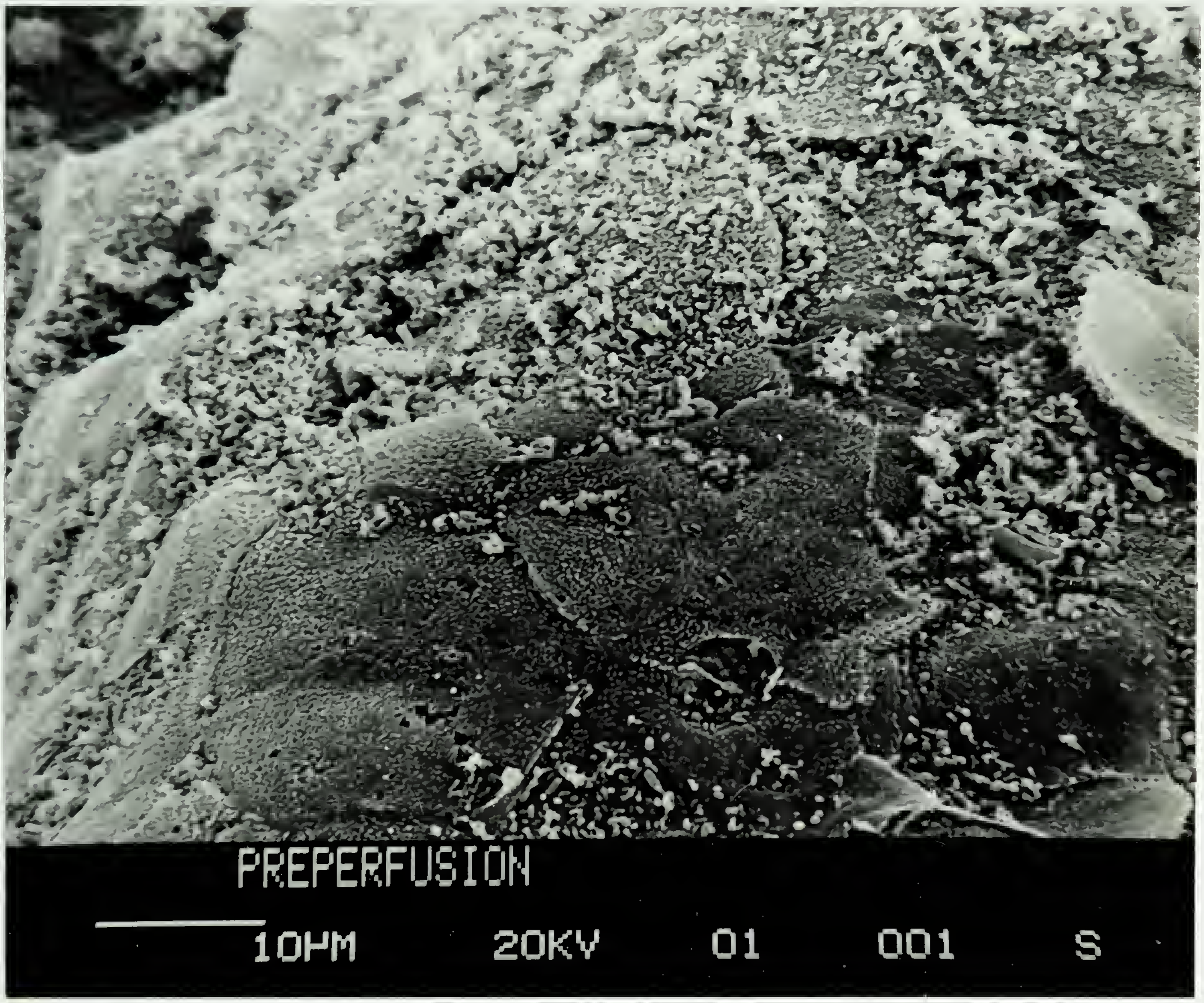

PREPERFUSION
10µM
20KV
01
001
S

Figure V.4 SEM of omasal epithelium, preperfusion. This micrograph depicts an area of epithelium with moderate coverage of adherent bacteria.

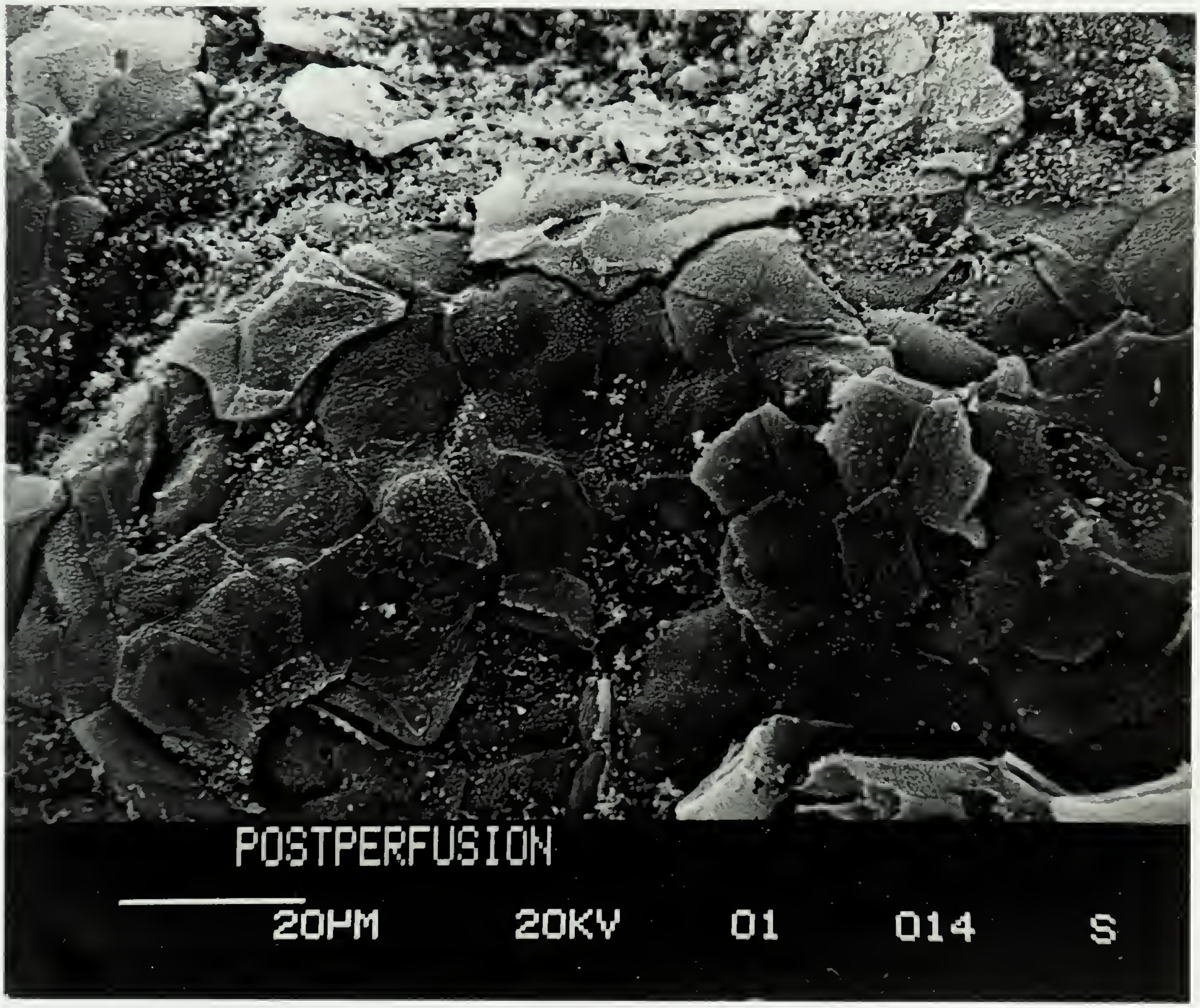

POSTPERFUSION
20µM
20KV
01
014
S

Figure V.5 SEM of omasal epithelium, postperfusion. This figure illustrates heavier colonization of microprotected areas (upper portion of figure) of the epithelium.

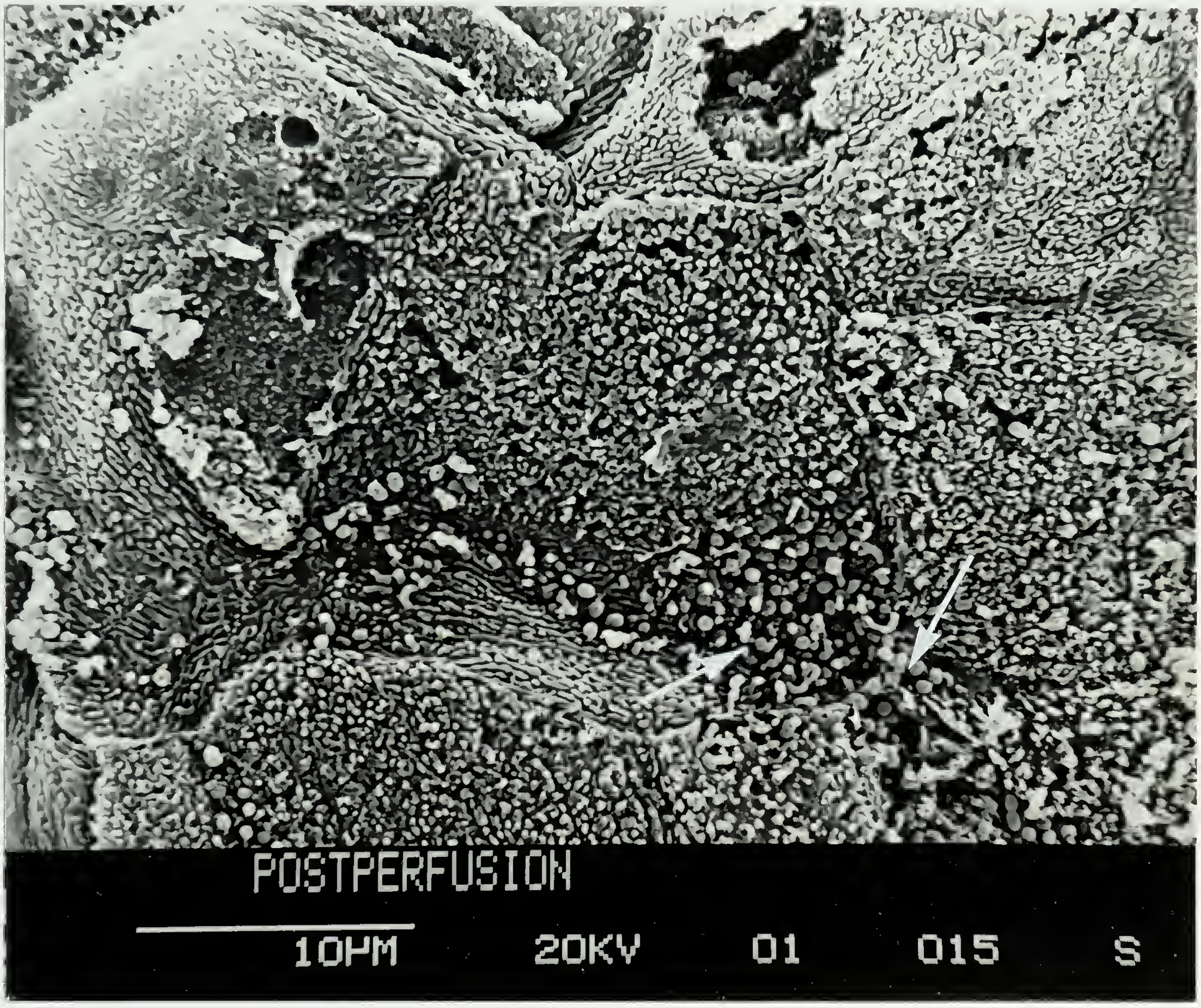
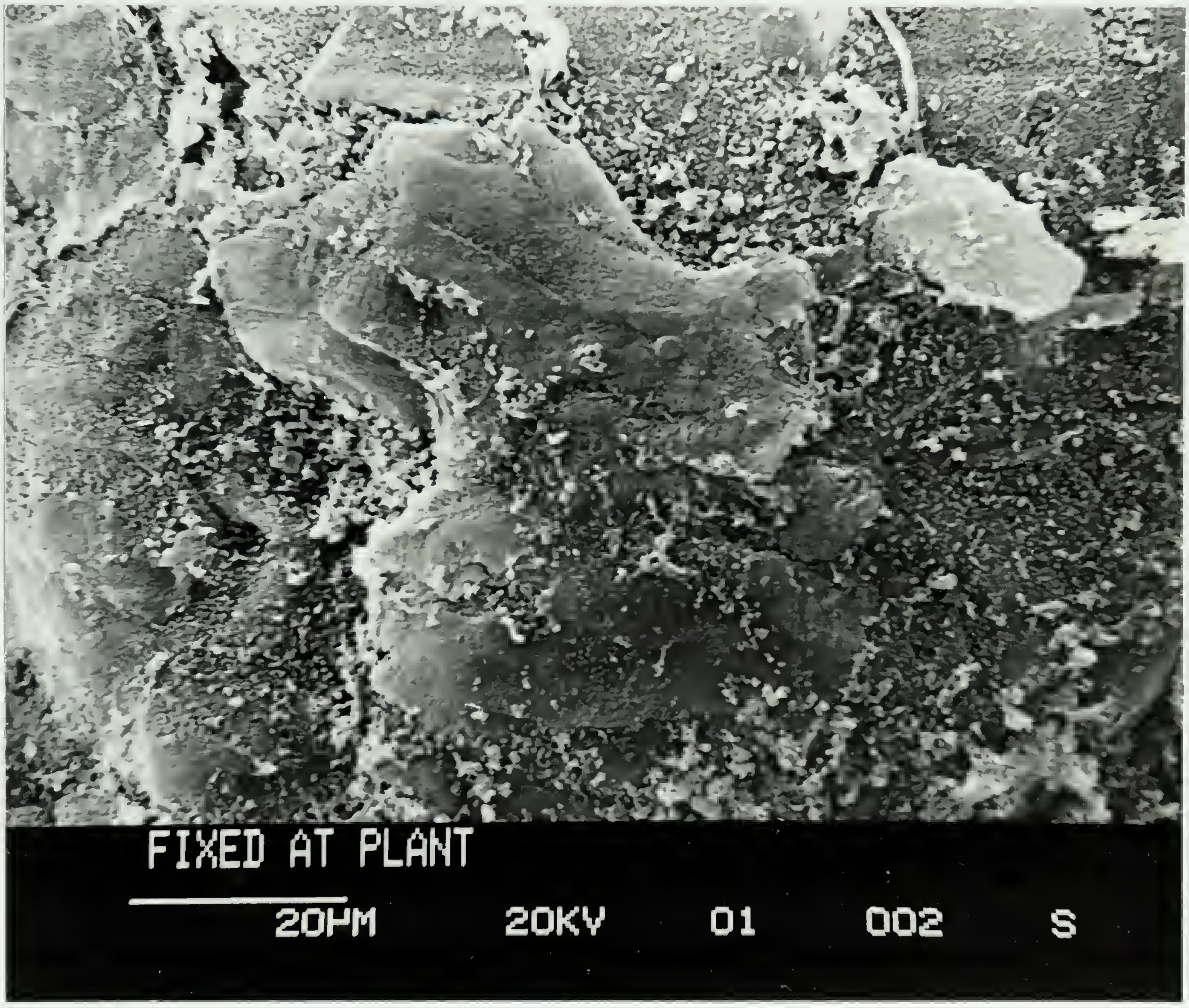

POSTPERFUSION
10PM
20KV
01
015
S

Figure V.6 SEM of omasal epithelium, postperfusion. The arrows point to areas of bacterial colonization. Other projections from the tissue are microvilli extending from the keratinized cells.


FIXED AT PLANT
20µM
20KV
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002
S

Figure V.7 SEM of omasal epithelium, fixed at abattoir. Moderate bacterial colonization of the epithelium, concentrated in microprotected areas.

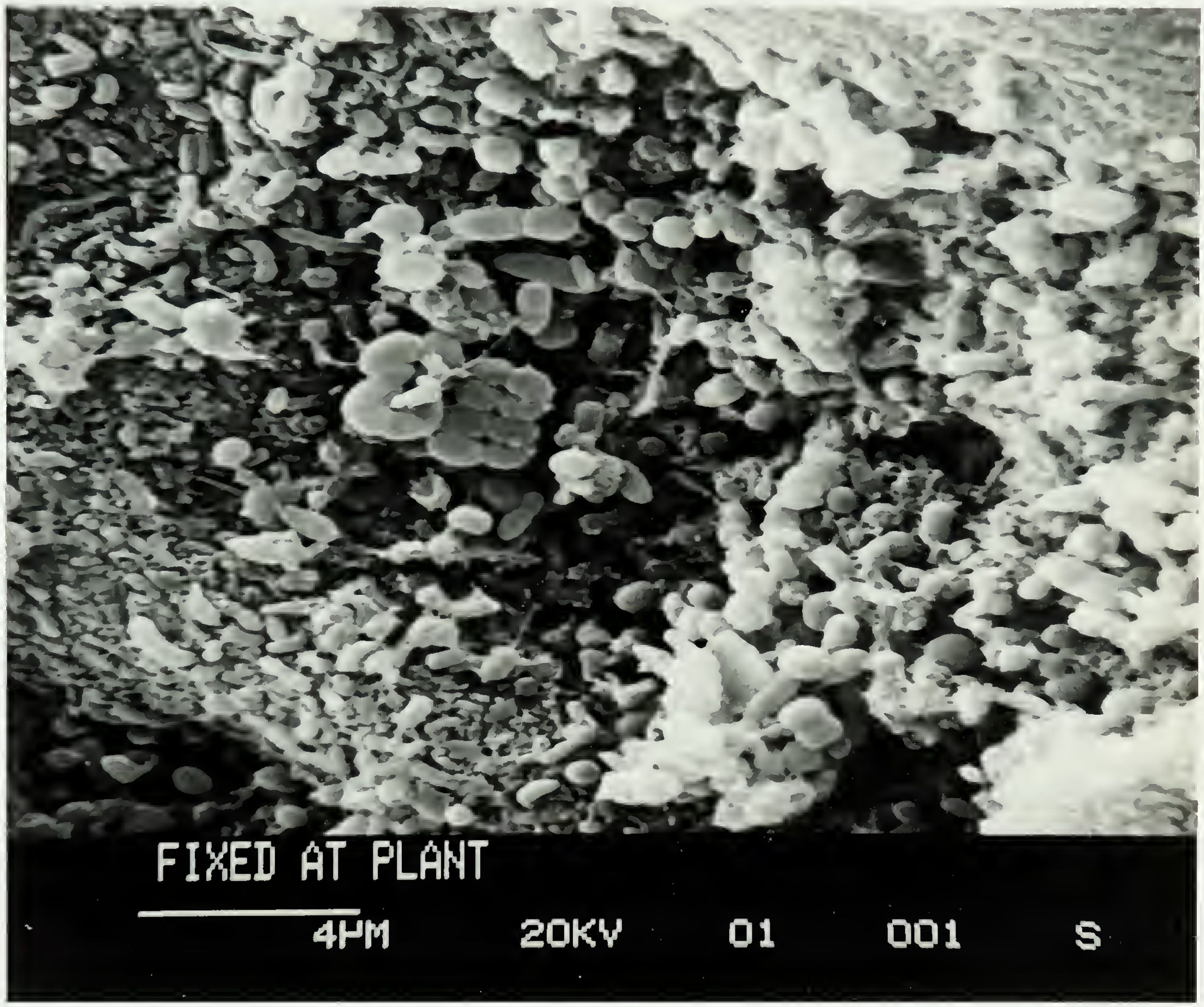
FIXED AT PLANT
4µM
20KV
01
001
S

Figure V.8 SEM of omasal epithelium, fixed at abattoir. High magnification micrograph illustrates heavy adherent population, some of which may be several layers in depth.

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APPENDIX I

This Appendix was constructed in order to provide an illustration of the steps involved in the catheterization of an omasal leaf and in the mounting of that tissue in the perfusion chamber.

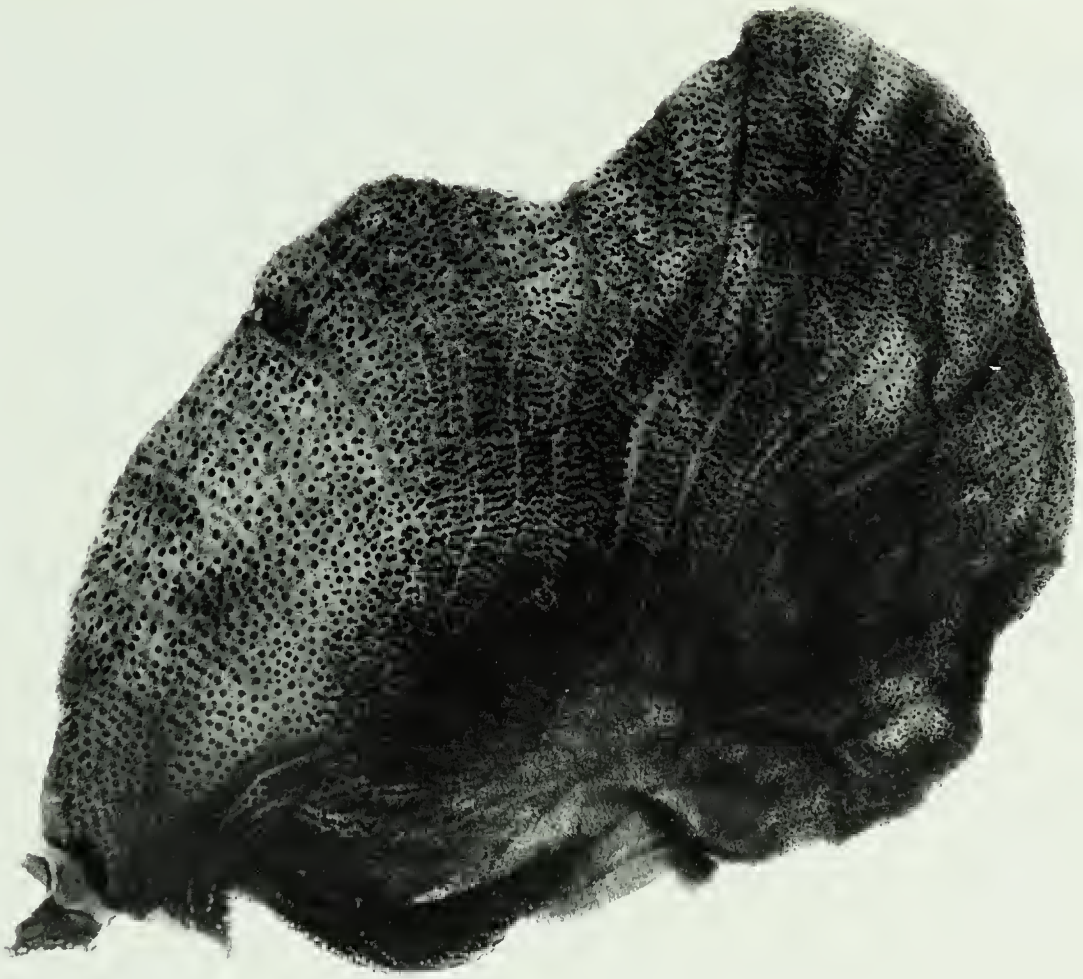

Figure 1. Bovine omasal leaf collected from a local abattoir. The convex boundary of the leaf is the edge cut away from the omasum. The faint outline of major vessels entering and exiting the base of the leaf are evident.

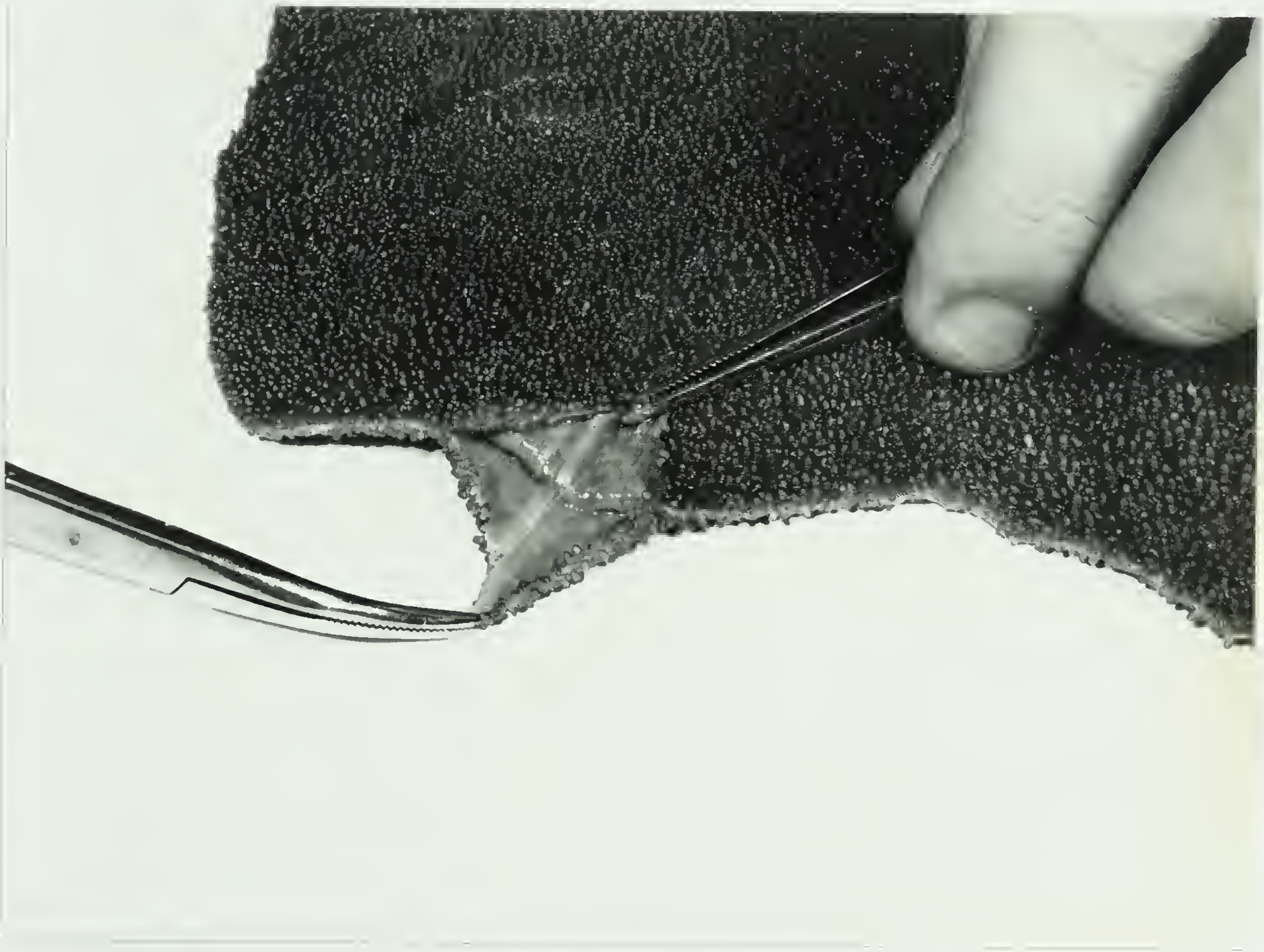

Figure 2. Exposure of the artery - vein couple to be catheterized. The laminae of the bovine omasum can be easily split, rendering the major artery - vein couple of the area exposed but undamaged and allowing relatively easy identification of the two vessels.

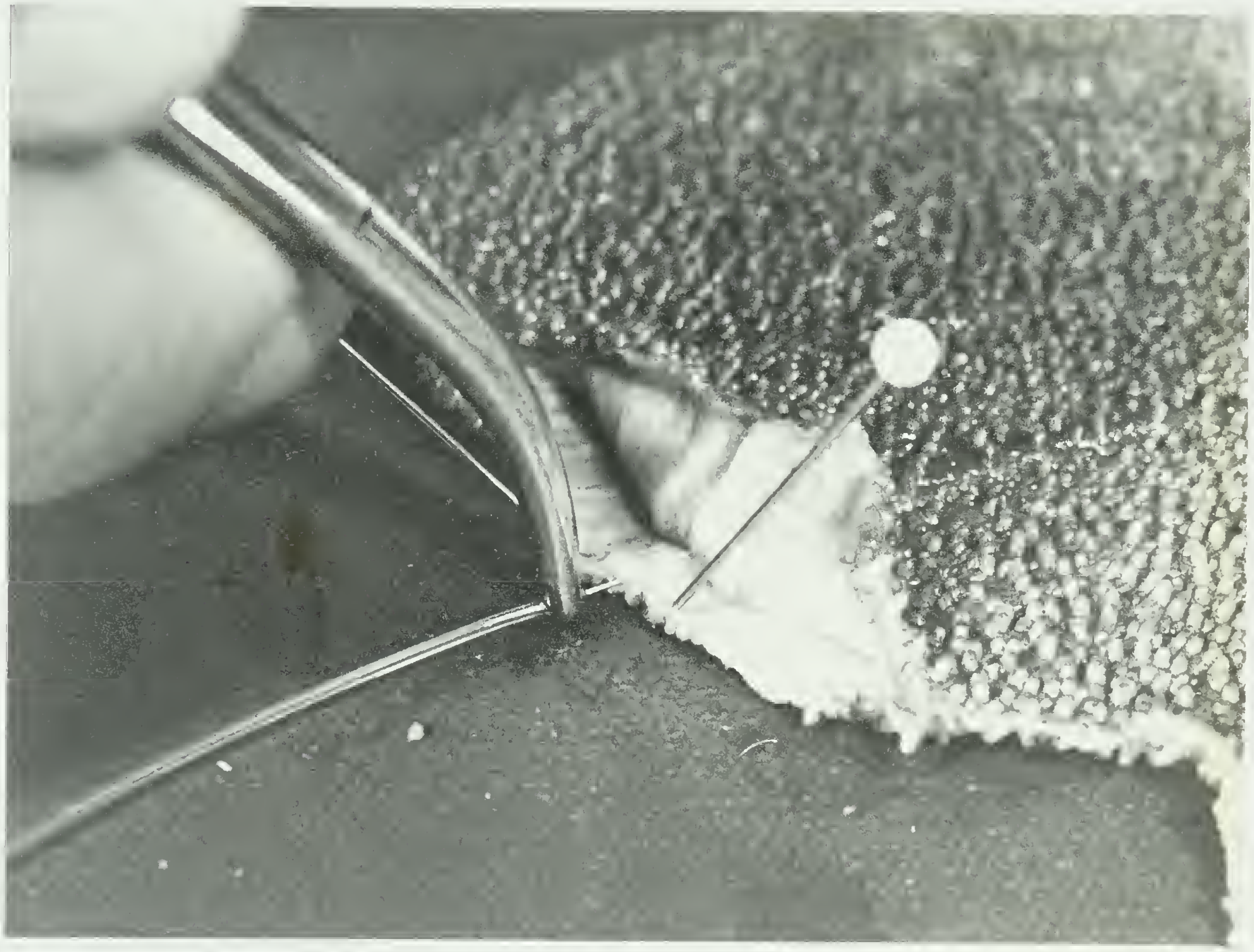

Figure 3. Insertion of the arterial catheter. Once exposed, the severed end of the artery and its satellite vein could be located and catheterized. Catheterization of the artery was accomplished under low magnification with a 0.152 mm I.D. X 0.305 mm O.D. stainless steel capillary tube (Small Parts Inc.) which was in turn connected to a length of polyvinyl chloride micro-bore tubing (0.254 mm I.D. X 0.762 mm O.D.; Cole Parmer).

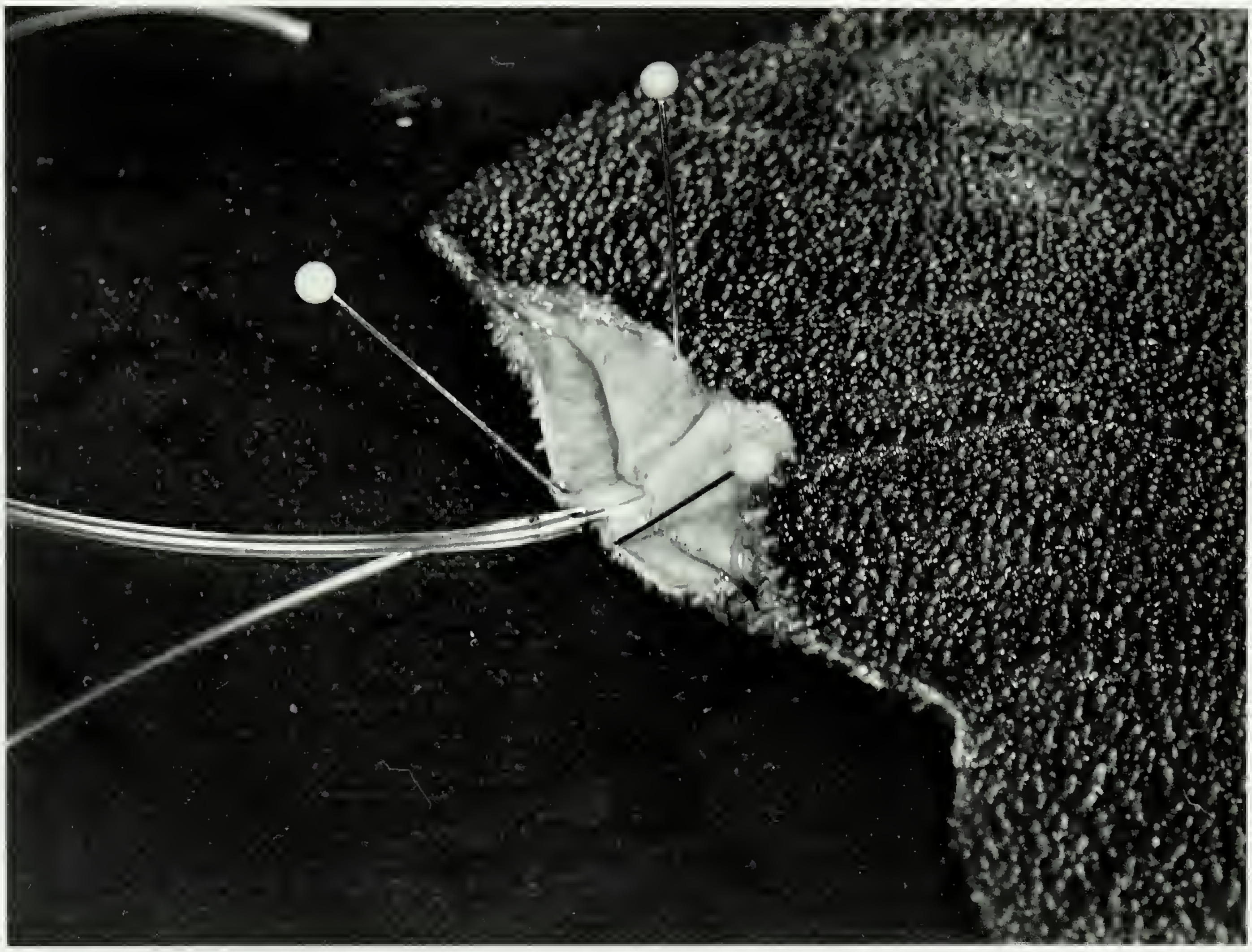

Figure 4. Arterial and venous catheters in position. Both catheters were inserted far enough into the vessels to ensure that the ends of the stainless steel tubes were embedded at least 0.5 cm into undisturbed tissue. The catheters were secured in this position by a single suture.

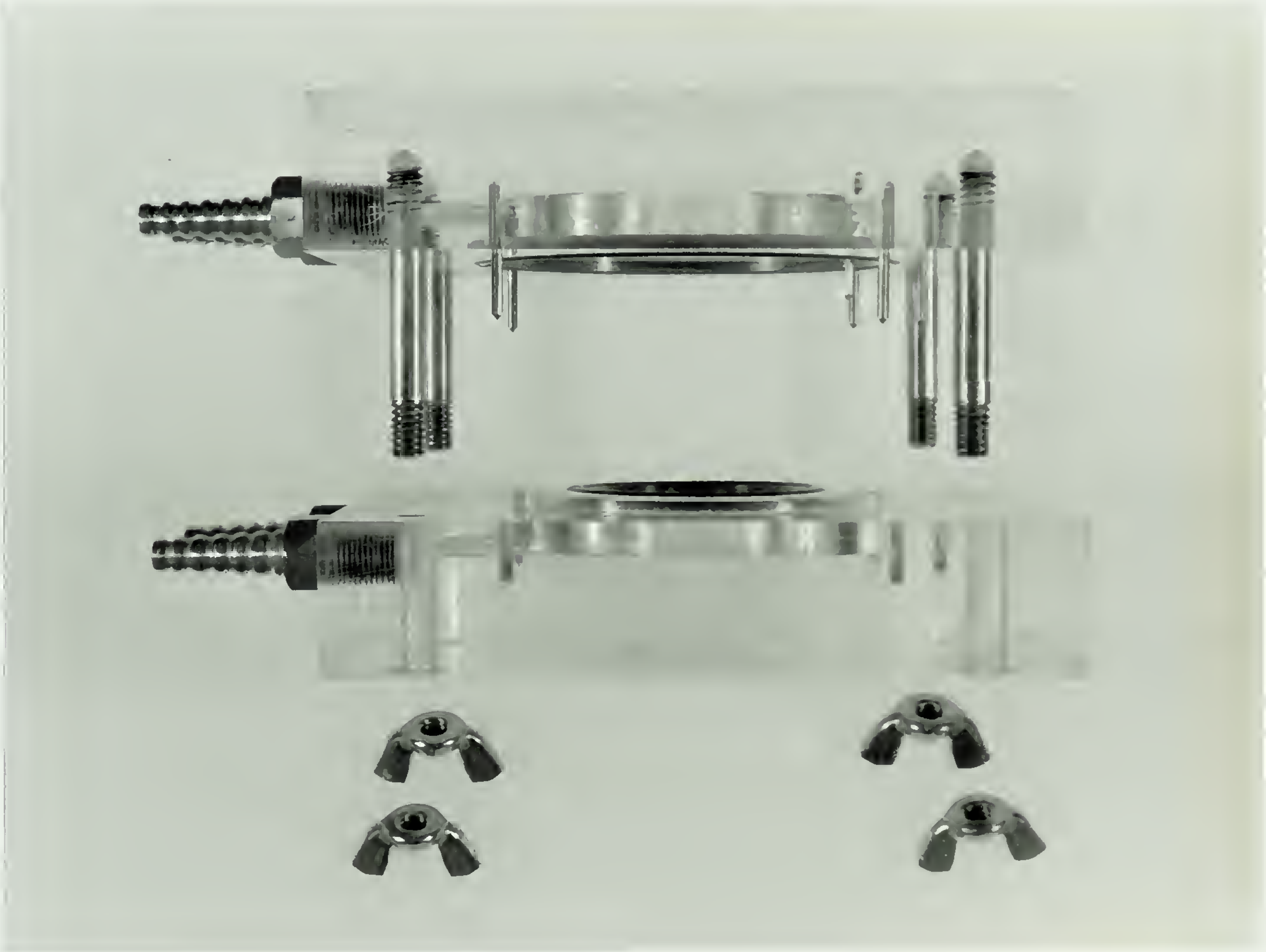

Figure 5. Side view of the two halves of the perfusion chamber. The perfusion chamber was designed in order to accomodate omasal leaf tissue and to effect isolation of a portion of that tissue. Dimensions of the persplex perfusion chamber are 12.5 X 10.0 X 0.8 cm (length X width X depth). Other features visible in this aspect include outlines of the lumen compartments and of the ports providing access to those compartments.

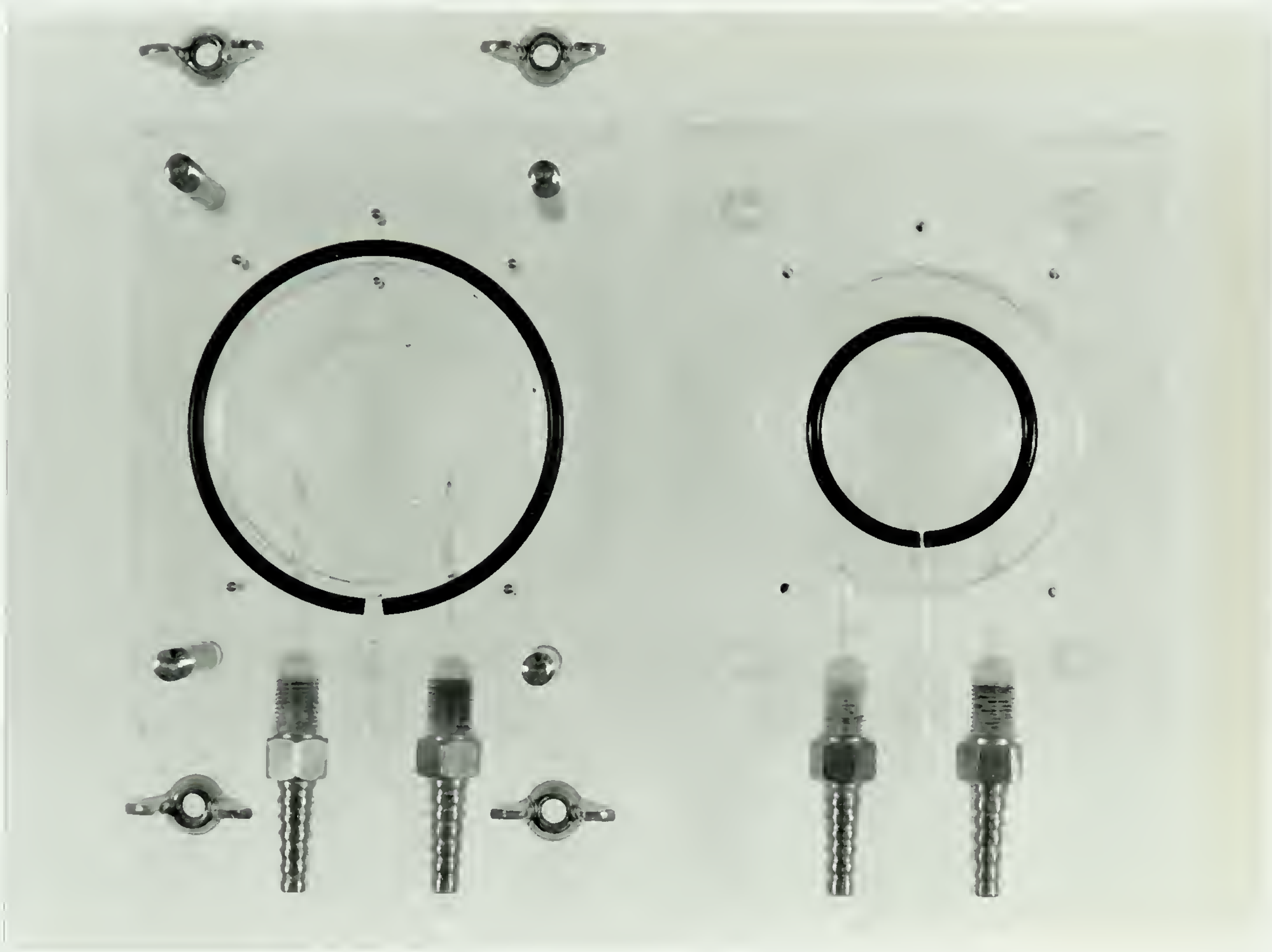

Figure 6. Top view of the two halves of the perfusion chamber. The O-ring in the base (right side) of the perfusion chamber effects the isolation of the perfusion area upon clamping. The outer O-ring, set into the other half of the chamber merely provides more efficient sealing of the chamber. Diameter of the lumen compartments is 3.7 cm. Lumen compartment access ports and the channel provided to prevent clamping of the catheters are visible.

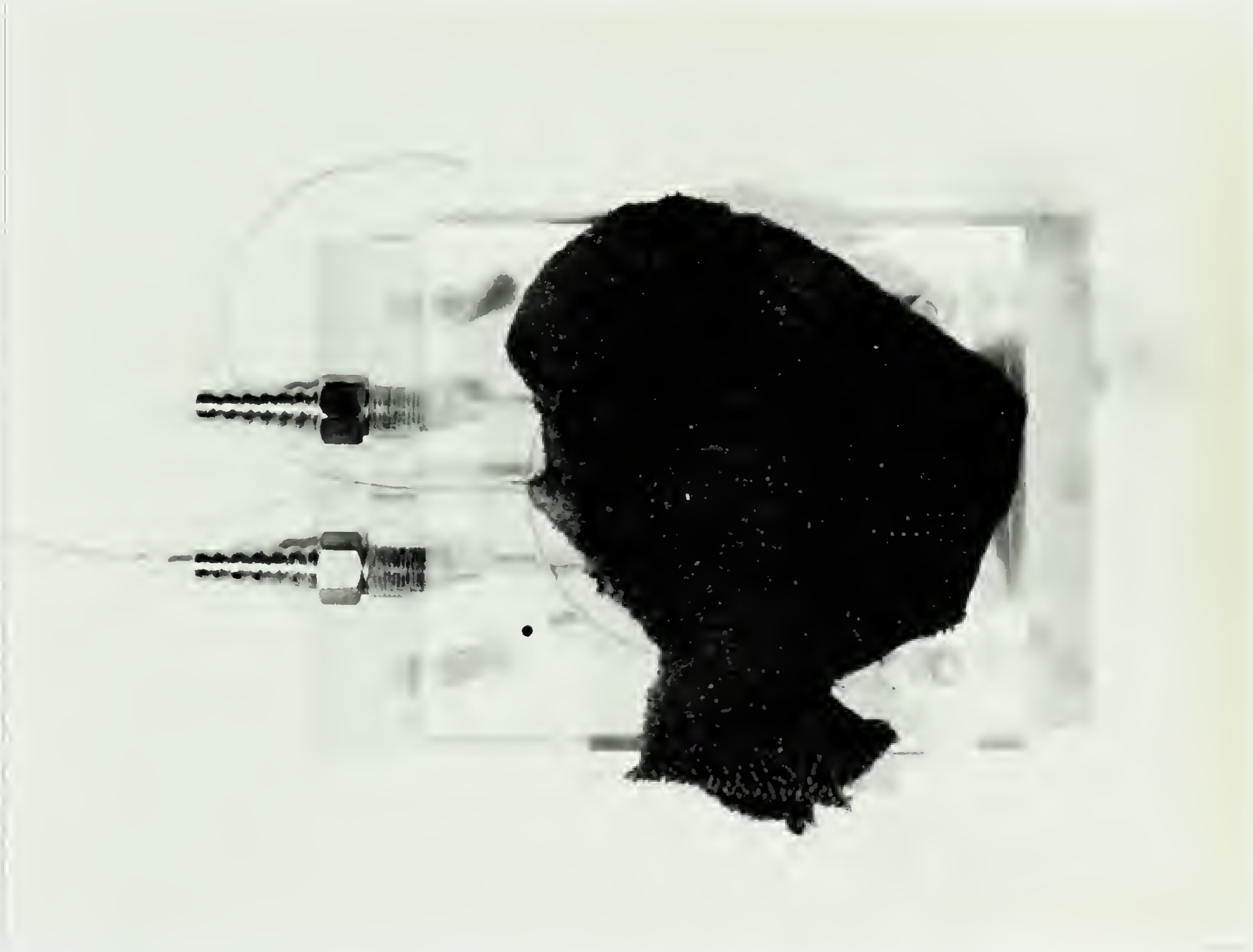

Figure 7. Omasal tissue on the base of the perfusion chamber. The tissue was situated on the base of the chamber so that the catheterized vessels ran down the center of the lumen compartments. The catheters were set in the channel in preparation of clamping.

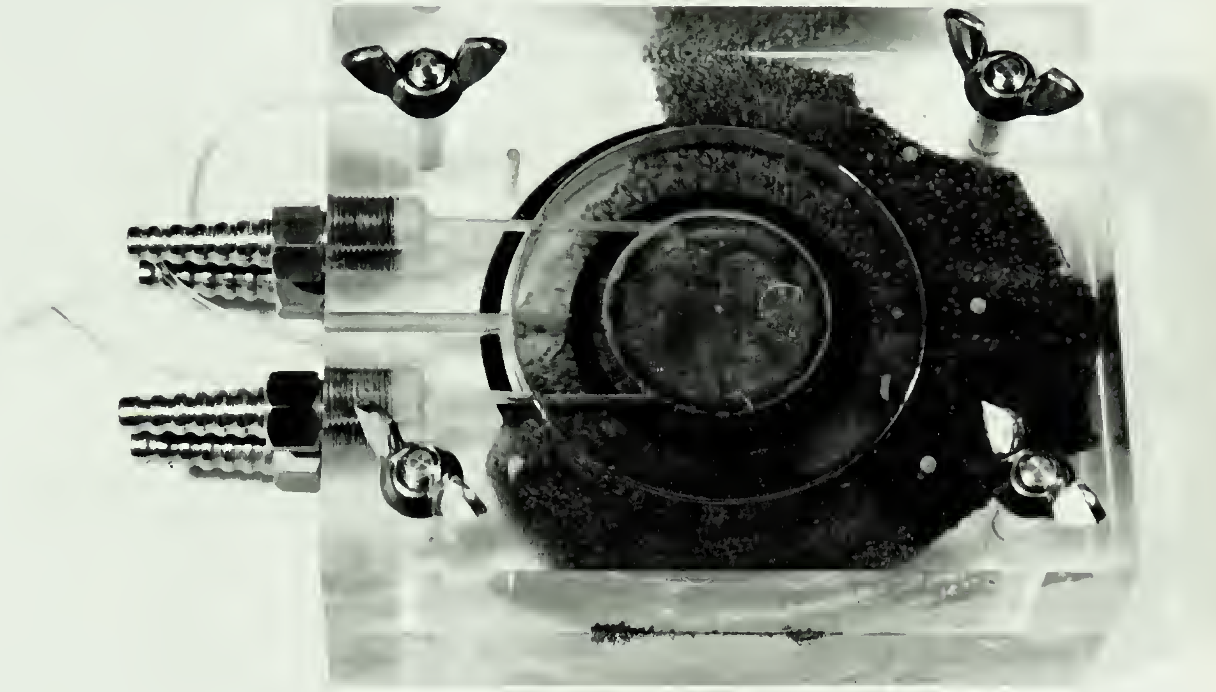

Figure 8. Mounted omasal tissue. The perfusion chamber was placed ports upward in a water bath (38 °C) when the tissue was properly secured and lumen solution placed in the lumen compartments. Water level in the bath was maintained just below the level where the catheters entered the tissue.

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